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NAME OF AUTHOR/*NOM DE L'AUTEUR* MARVIN ORVILLE SALOMONS

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NAME OF SUPERVISOR/*NOM DU DIRECTEUR DE THÈSE* DR. F.X. AHERNE DR. L.P. MILLIGAN

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EFFECTS OF DIETARY PROPIONIC ACID ON PLASMA AND TISSUE CHOLESTEROL LEVELS IN SWINE

by

© MARVIN O. SALOMONS

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES

AND RESEARCH IN PARTIAL FULFILMENT OF THE

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FACULTY OF GRADUATE STUDIES AND RESEARCH

The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research, for acceptance, a thesis entitled "Effects of dietary propionic acid on plasma and tissue cholesterol levels in swine" submitted by Marvin O. Salomons, in partial fulfilment of the requirements for the degree of Master of Science in Animal Nutrition.

Francis Aherne
Supervisor

Dated June 10, 1977

ABSTRACT

In 5 experiments, a control diet (C) or diets containing 10 or 15% tallow (T) with or without the addition of propionic acid (PA) were fed to growing swine to determine the effects on plasma and tissue cholesterol levels. Cholesterol (CH) rapeseed oil (RSO) and sugar (S) were also added to swine diets to test their hypercholesterolic effects. Pig performance as measured by average daily gain (ADG), average daily feed (ADF), and efficiency of feed conversion (EFC) was also determined. Digestion studies were also conducted in Experiment 1. Blood collection and sampling methods, the effects of starvation on plasma cholesterol and the relationship of plasma total lipid to plasma total cholesterol was also measured. A cholesterol inhibitor (AY-9944) was used to determine the rate of cholesterol synthesis in pigs fed 15%T and 15%T + 5%PA diets.

The addition of T or RSO caused a reduction in ADF and an improvement in ADG and EFC. The inclusion of PA in the diet improved EFC, but ADG and ADF were slightly less than those of pigs fed the C diet. There were no significant differences in % digestible energy and % digestible nitrogen in pigs fed C, 5%PA, 10%T or 10%T + 5%PA diets.

The inclusion of 5%PA in the diet tended to depress plasma cholesterol levels while the addition of tallow tended to increase plasma cholesterol levels. The increase in plasma cholesterol obtained with 10%T was about half that observed when feeding 15%T. The inclusion of cholesterol

or sugar had no significant effect on plasma cholesterol levels. No significant differences in plasma cholesterol between males and females were observed in any of the experiments. The inclusion of 10%T in the diet increased the cholesterol content of bile and tissue samples. The inclusion of 5%PA both with and without 10%T significantly ($P < 0.05$) lowered kidney cholesterol content. There were no significant differences in cholesterol contents of liver, back muscle, leg muscle and fat, and bile.

Blood sampling and collection methods and 12 hours starvation prior to bleeding had no effect on blood cholesterol concentrations. Plasma total lipid content had no significant influence on the determination of plasma total cholesterol ($r^2 = 0.32$). Propionic acid in the diets of pigs appeared to have a significant effect on cholesterol synthesis in the liver, as large differences ($P < 0.001$) were observed in the levels of plasma 7-dehydrocholesterol of pigs 24 and 48 hours after being orally dosed with the compound AY-9944 (Experiment 5).

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TABLE OF CONTENTS

	PAGE
INTRODUCTION	1
LITERATURE REVIEW	3
1. Lipids and Atherosclerosis	3
2. Controls of Cholesterol Synthesis	7
3. Factors Affecting Cholesterol Levels and Incidence of Atherosclerosis	9
a. Fat, Cholesterol and Protein	9
i. Studies with swine	9
ii. Studies with other animals	15
4. Cholesterol Values in Pigs	16
a. Age and Sex Effects	18
5. Influence of Dietary Fat or Oil on Pig Performance	19
a. Growth Rate and Feed Intake	19
b. Digestibility of Fat or Oils in the Diet	21
6. The Influence of Dietary Volatile Fatty Acids on Pig Performance	22
a. Growth Rate and Feed Intake	22
7. Propionate Metabolism	23
a. The Effect of Propionate on Ketogenesis	23
b. The Effect of Propionate on Cholesterolgenesis	24
CONDITIONS COMMON TO ALL EXPERIMENTS	27
1. General Objectives	27
2. Experimental Procedures	27

PAGE

STATISTICAL ANALYSIS 29

METHODS OF CHEMICAL ANALYSIS 30

1. Feed and Feces Samples 30

2. Plasma Total Cholesterol 30

3. Tissue Total Cholesterol 31

4. Determination of Cholesterol in Bile 32

5. Estimation of Total Plasma Lipids 32

6. Determination of Fatty Acid Patterns of Tallow and Rapeseed Oil Samples 33

7. Determination of 7-dehydrocholesterol 33

EXPERIMENT 1 34

1. Objective 34

2. Experimental Procedures 34

a. Animals and diets 34

b. Collection of samples 34

i. Blood samples 34
ii. Tissue samples 36
iii. Bile samples 36

3. Digestion Study 36

Results and Discussion 39

1. Pig Performance; average daily gain (ADG) average daily feed intake (ADF) and efficiency of feed conversion (EFC) 39

2. Digestibility Studies 41

3. Blood Studies 41

4. Tissue and Bile Studies 45

Summary 49

PAGE

EXPERIMENT 2 51
1. Objectives 51
2. Experimental Procedures 51
a. Animals and diets 51
b. Collection of blood samples 51
Results and Discussion 53
1. Pig Performance (ADG, ADF, and EFC) 53
2. Blood Studies 53
Summary 55
EXPERIMENT 3 57
1. Objectives 57
2. Experimental Procedures 57
a. Animals and diets 57
b. Collection of samples 59
Results and Discussion 60
1. Pig Performance (ADG, ADF and EFC) 60
2. Blood Studies 60
3. Blood Sampling and Collection Study 63
Summary 65
EXPERIMENT 4 66
1. Objectives 66
2. Experimental Procedures 66
a. Animals, diets and blood collection 66
Results and Discussion 69
1. Blood Studies 69
2. Relationship of Plasma Cholesterol and
Plasma Lipid Content 71

PAGE

3. Starvation Study 73

Summary 75

EXPERIMENT 5 77

1. Objectives 77

2. Experimental Procedures 77

a. Animals, diets, and blood collection . . . 77

b. Dosing with AY-9944 79

Results and Discussion 80

1. Pig Performance 80

2. Plasma 7-Dehydrocholesterol Studies 80

3. Plasma Total Cholesterol and Profile Studies . 82

Summary 85

GENERAL DISCUSSION 86

BIBLIOGRAPHY 91

APPENDIX A DETERMINATION OF TOTAL CHOLESTEROL IN BILE 101

APPENDIX B ESTIMATION OF TOTAL LIPIDS IN BLOOD PLASMA 102

APPENDIX C DETERMINATION OF FATTY ACID PATTERNS IN TALLOW OR OIL 104

Fatty Acid Composition of Tallow and Rapeseed Oil Samples 105

APPENDIX D DETERMINATION OF 7-DEHYDROCHOLESTEROL . . 107

LIST OF TABLES

Table	Description	Page
1	Typical Fatty Acid Analyses of Some Fats of Animals and Plant Origin	4
2	Summary: Blood Cholesterol Levels in Swine	17
3	Formulation and Composition of Diets Experiment 1	35
4	Composition of Diets for Digestibility Trials Experiment 1	38
5	Performance of Pigs from 0 to 10 weeks on Experiment	40
6	Nitrogen and Energy Digestibility Data (%)	42
7	Plasma Cholesterol Concentrations from 0 to 10 Weeks on Experiment	44
8	Tissue and Bile Cholesterol Concentrations (Pre- and Post-Treatment)	46
9	Formulation and Composition of Diets Experiment 2	52
10	Performance and Plasma Cholesterol Concentrations of Pigs	54
11	Formulation and Composition of Diets Experiment 3	58
12	Performance and Plasma Cholesterol Concentrations of Pigs	61
13	Method of Blood Sampling on Blood Cholesterol Concentrations (mg/100 ml)	64
14	Formulation and Composition of Diets Experiment 4	67
15	Plasma Cholesterol Concentrations of Pigs on Experiment	70
16	Relationship of Total Cholesterol and Total Lipid in Plasma	72

Table	Description	Page
17	Effect of Starvation on Total Plasma Cholesterol Concentrations	74
18	Formulation and Composition of Diets Experiment 5	78
19	Performance and Blood 7-Dehydrocholesterol Concentrations of Pigs Dosed or Not Dosed with AY-9944	81
20	Plasma Total Cholesterol (0, 24 and 48 hours) and Plasma Constituents (48 hours)	83
21	Fatty Acid Patterns of Tallow and Rapeseed Oil in Percentage by Weight of Fatty Acids	106

LIST OF FIGURES

Figure		Page
1	Schematic Representation of Cholesterol and Ketone Body Formation in Liver	25

INTRODUCTION

Cholesterol occupies a role of significant importance in the animal body. Excessive levels of cholesterol, as well as other lipids, in the circulatory system of many animals have been shown to cause a disease termed "atherosclerosis". Atherosclerosis is characterized by an accumulation of soft, amorphous lipids (including cholesterol) in the arterial walls causing the formation of intimal plaques or lesions. If the lesions enlarge they may hemorrhage, reduce the size of the lumen, or possibly completely occlude the blood vessel. Although this condition has been shown to occur in many animals, it is of primary importance to man since hundreds of thousands of people die each year of this disease.

Dozens of factors have been suggested to be contributors to the occurrence of this disease. Among those receiving most frequent mention are heredity, stress, obesity, hypertension, diabetes mellitus, cigarette smoking, lack of exercise, diet and hypercholesterolemia (Jacobson, 1974). Although there is still considerable disagreement regarding the pathogenesis of the disease, considerable progress has been made in our knowledge of the disease and in its' prevention and cure. The importance of controlling hypercholesterolemia in man has received considerable attention. Reducing circulating levels of cholesterol by either dietary means or by controlling rates of cholesterol

synthesis have been successful to only a minor degree. The level of fat in the diet has been implicated as being one of the main factors in causing high blood cholesterol levels therefore controlling its intake has become one of the prime methods of reducing blood cholesterol levels.

Bush and Milligan (1971) reported that the formation of ketone bodies could be reduced through the partial inhibition of the enzyme, beta-hydroxymethylglutaryl-CoA synthase, which is part of a common pathway involving both the formation of ketone bodies and cholesterol. Evidence suggested that propionyl-CoA competition with acetyl-CoA could possibly result in inhibition of the enzyme and thereby reduce the rates of tissue cholesterol synthesis and hence the level of blood cholesterol.

The use of the pig has become exceedingly popular in research dealing with "atherosclerosis" because the responses associated with this disease in man also occurs in the pig. The studies described herein were undertaken to determine the effects of propionic acid in diets of pigs on plasma and tissue cholesterol levels or its rate of synthesis.

LITERATURE REVIEW

1. Lipids and Atherosclerosis

The lipids are a heterogeneous group of compounds which include fats, oils, waxes, phospholipids, glycolipids, steroids and related substances. Fats and oils comprise by far the largest group of lipids and yield fatty acids and glycerol upon hydrolysis. Fatty acids which occur in natural fats usually contain an even number of carbon atoms (because they are synthesized from 2-carbon units), are straight chain derivatives, and may be saturated (containing no double bonds) or unsaturated (containing one or more double bonds). Fats or oils are considered to be saturated or unsaturated depending on the degree of saturation of the component fatty acids. The fatty acid contents of some animal fats and vegetable oils are shown in Table 1. In general, the animal fats are considered to be saturated even though their unsaturated fatty acid content may be quite high. On a weight basis, beef fat contains approximately 60% saturated fatty acids and pig and chicken fat less than 50% and 40% respectively (Jacobson, 1974). Although most vegetable oils contain higher levels of unsaturated fatty acids than do animal fats there is one notable exception, coconut oil, in which only 10% of the fatty acids are unsaturated.

TABLE 1

TYPICAL FATTY ACID ANALYSES OF SOME FATS OF ANIMAL AND PLANT ORIGIN[1]

	SATURATED			UNSATURATED		
	Palmitic	Stearic	Other	Oleic	Linoelic	Other
Animal Fats						
Lard	29.8	12.7	1.0	47.8	3.1	5.6
Chicken	25.6	7.0	0.3	39.4	21.8	5.9
Butterfat	25.2	9.2	25.6	29.5	3.6	7.2
Beef Fat	29.2	21.0	3.4	41.1	1.8	3.5
Vegetable Oils						
Corn	8.1	2.5	0.1	30.1	56.3	2.9
Peanut	6.3	4.9	5.9	61.1	21.8	--
Cottonseed	23.4	1.1	2.7	22.9	47.8	2.1
Soybean	9.8	2.4	1.2	28.9	50.7	7.0[2]
Olive	10.0	3.3	0.6	77.5	8.6	--
Coconut	10.5	2.3	78.4	7.5	trace	1.3

[1] From Mayes, 1973. All values in weight percentages of component fatty acids.

[2] Mostly linolenic acid.

The group of lipid derivatives containing three fused cyclohexane rings in the phenanthrene arrangement with 8 to 10 carbon atoms in the side chain at position 17 and an alcoholic hydroxyl group at position 3 are classed as sterols, of which the most abundant representative in animal tissues is cholesterol. The tissues in which cholesterol is most frequently found are the body fats, bile, brain, liver, kidney, nerve tissue, blood, skin, arteries and egg yolk. It is a necessary part of cellular membranes, is necessary for the production of sex and adrenal hormones, and is a precursor in the formation of bile acids. Acetyl units are the basic building blocks in cholesterol synthesis. The more acetyl units that are made available, for example, from feeding large amounts of dietary fat, the greater the rate of synthesis of cholesterol (Bortz, 1973).

High dietary fat levels depress fatty acid synthesis from acetate because many of the fatty acids needed for the production of body fat are derived from the diet under these conditions (Naber, 1976). The need for acetate for fatty acid synthesis is reduced making the acetate pool available for energy production or cholesterol formation. Huang and Kummerow (1976) observed in studies with swine tissues that when acetate was available; liver, small intestine, and adipose tissue were important sites of cholesterol synthesis, but that the main site of plasma cholesterol synthesis in swine was the liver. The acetate obtained from endogenous or exogenous sources would in these tissues be available for cholesterol synthesis. The continued need for cholesterol

in the body may be satisfied by endogenous synthesis or obtained from exogenous sources. The greater part of cholesterol in the human body arises by net synthesis (about 1 g/day) whereas only about 0.3 g/day is provided by the average diet (Mayes, 1973). However other estimates have placed the average cholesterol intake in man to be about 1 g/day, with a range of 0.5 to 2.0 g (Taylor and Ho, 1967).

Over 20 years ago (Keys, 1953) reported that a positive correlation existed between the level of dietary fat or dietary cholesterol and serum cholesterol levels, and all these appeared to be related to the presence of cholesterol containing lesions (plaques) in the arterial system of man and animals. The increasing occurrence of such lesions in man, termed "atherosclerosis", has led many researchers to studies of how the disease can be prevented or controlled. The use of swine in research of problems associated with excessive circulation of cholesterol in the arterial system has become increasingly common. There appears to be little doubt that the lesions occurring in the aortas and coronary arteries of swine resemble uncomplicated human atherosclerosis and that incident to the development of severe lesions consistent elevations in both plasma and serum cholesterol levels have been observed (Moreland et al, 1963; Kummerow et al, 1974.

2. Controls of Cholesterol Synthesis

In most species of animals cholesterol synthesis is controlled via a feed-back mechanism and therefore can be influenced by the addition of dietary cholesterol (Wilson et al, 1967; Naber, 1976). Although some animals do not have as effective a control mechanism as others, in most species feed-back control occurs by inhibition of the enzyme β-hydroxymethylglutaryl-CoA (HMG-CoA) reductase (Myant, 1975; Sabine and James, 1976). Another hypothesis is that there is an intestinal factor capable of stimulating or inhibiting hepatic cholesterol synthesis whenever there is a need for increased amounts of bile acids, (Krumdieck and Ho, 1977). In a review, Bortz (1973) mentioned a number of factors such as fat feeding, cholesterol feeding or bile acid feeding which may influence hepatic cholesterol synthesis but he suggested that the HMG-CoA reductase step was probably the most important site for the control of cholesterol synthesis.

The suggested mechanisms of maintaining cholesterol balance in the body have been outlined (Naber, 1976). Naber stated that the blood and tissue levels of cholesterol are determined by the balance achieved between dietary inputs and body synthesis on one hand, and excretion of neutral sterols and oxidation to bile acids on the other hand. Studies of cholesterol turnover, synthesis and retention have been conducted with swine under hypercholesterolemic conditions,

(Marsh et al, 1972). This study evaluated the rates of synthesis and absorption of cholesterol in pigs fed either a milk-cholesterol diet or a mash-cholesterol diet. Absorption of cholesterol and plasma cholesterol levels were significantly greater in pigs fed milk-cholesterol diets whereas synthesis of cholesterol was greatest in animals fed the mash-cholesterol diets. Their study also showed that the accumulation of cholesterol in most body tissues of growing pigs was directly related to an increase in body size.

Kekki et al (1977) reported that in many animal species including man, the rates of cholesterol synthesis have been determined using labelled cholesterol or fecal steroid analysis. One unique and useful assay for liver cholesterol synthesis involves the use of a cholesterol inhibitor AY-9944 (trans-1, 4-bis (2 chlorobenzylaminomethyl) cyclohexane dihydrochloride) (Humber 1963; Dvornik et al, 1963) which has allowed an estimation of the rates of cholesterol synthesis in rats (Horton et al, 1971). Dvornik et al, (1965) suggested that AY-9944, which is an inhibitor of cholesterol biosynthesis, could be used to estimate cholesterol synthesis by measuring the amount of 7-dehydrocholesterol (precursor) formed over a certain period of time.

3. Factors Affecting Cholesterol Levels and Incidence of Atherosclerosis

a. Fat, Cholesterol and Protein

i. Studies with swine:

The addition or change of certain dietary components have been shown to be important factors contributing to increasing blood cholesterol levels and in the development of lesions of atherosclerosis. The level or type of fat or oil in the diet, and the dietary level of cholesterol and protein have been studied extensively to determine their relationship to cholesterol concentrations in the blood and tissues. The literature reviewed in this thesis will deal primarily with studies involving swine.

A greater incidence of atherosclerotic lesion development in pigs was reported from feeding saturated fats as opposed to feeding unsaturated fats in the diet. Howard et al, (1965) fed to growing pigs a commercial diet or three semisynthetic diets containing no-fat, 10% beef tallow, or 10% maize oil. Serum cholesterol levels were variable but the mean values for each diet were not significantly different. Their results showed that serum cholesterol concentrations were 10% lower in the tallow fed group in comparison to the levels obtained with pigs fed the maize oil diets. The results confirm the observation that animals fed the unsaturated fat have lower mean serum cholesterol levels than those fed saturated fat. Pigs fed fat or oil

supplemented diets had higher serum cholesterol values than pigs fed the commercial or the no-fat semisynthetic diets. This study also demonstrated that feeding semisynthetic diets to pigs did not increase the concentration of serum cholesterol which is in contrast to earlier results obtained with rabbits (Wigand 1960; Gresham and Howard, 1962).

Peifer and Lundberg (1957) studied the influence of specific fatty acids in the diets of minature pigs and found that lower cholesterol levels were observed in pigs fed unsaturated fatty acids. Subsequently, Peifer and Lundberg (1958) attempted to evaluate simultaneously the influence of total calories, fat calories and degree of fat unsaturation on the levels of blood cholesterol. Minature pigs fed diets with 18% fat (beef tallow or corn oil) had plasma cholesterol levels twice those of pigs fed 5.6% fat in their diets. Source of fat had no significant effect on blood cholesterol levels but corn oil tended to give lower cholesterol values. These authors also suggested that the calorie intake as fat is the most important factor influencing blood cholesterol level rather than total calorie intake. A more recent study by Hutagalung et al, (1969) showed that pigs fed either 5% lard, beef tallow or sheep tallow had significantly higher serum, muscle and liver cholesterol levels than pigs fed control diets. These trials also demonstrated that cholesterol levels were significantly higher in pigs fed animal fats (lard, beef tallow or sheep tallow) compared with pigs fed corn oil. Pigs fed tallow had higher

serum cholesterol levels than those fed lard. Hill et al (1971a) reported that the pigs fed coconut oil had mean serum cholesterol values considerably lower than those of pigs fed tallow. They also noted that the serum cholesterol levels of pigs fed coconut oil were not as markedly elevated during the first weeks of the experiment as those of pigs fed tallow. A recent study by Aherne et al (1976) demonstrated that high serum cholesterol values were obtained in growing pigs fed diets containing 15% high or low erucic acid rapeseed oils. Cholesterol levels of blood samples taken after 4 weeks (18.7 kg) on the experiment were significantly higher for pigs fed the rapeseed oil containing diets than for pigs fed the control diets. No significant differences between dietary treatments were reported at 16 weeks (87 kg) or 23 weeks (130 kg) of the experiment.

Dietary fat plus added cholesterol has also been shown to increase blood cholesterol levels and promote the development of atherosclerotic lesions in both man and animals. Keys et al (1956) suggested that, in man, dietary cholesterol is of lesser importance than dietary fat in influencing blood cholesterol levels and atherosclerotic lesion development. However, Connor (1968) concluded that dietary cholesterol is the most important factor contributing to increased blood cholesterol and the promotion of an atherosclerotic disease condition.

Gyorkey and Reiser (1964) determined the extent to which atherosclerosis could be induced in young minature swine. The study consisted of feeding diets containing 20%

saturated fat (myristoyllaurin) or unsaturated fat (cottonseed oil) alone or in combination with 2% cholesterol. They reported that only swine fed the saturated or unsaturated fats plus cholesterol diets developed gross and microscopic atherosclerotic lesions. Plasma and liver cholesterol levels were higher for swine fed either type of fat compared to control animals but the highest plasma cholesterol values were obtained with pigs fed the unsaturated fat (77 mg/100 ml) as compared with those fed the saturated fat (60 mg/100 ml). They suggested that these results reflect the influence of saturated fatty acids on cholesterol absorption.

Greer et al (1966) studied a combination of effects of high and low levels of energy intake, two fat sources (soybean oil or tallow), protein level (18% versus 12%), and the addition of 1% cholesterol in pig diets on serum cholesterol levels. They also observed the effects on the incidence of atherosclerotic lesions in the thoracic and abdominal aortas and coronary arteries. Feeding 15% tallow resulted in higher serum cholesterol levels than observed with feeding 15% soybean oil. The tallow supplemented diets also resulted in an increased incidence of arterial lesions, except in one trial where pigs fed the soybean oil diets had a greater incidence of lesions. The level of energy intake was not important in influencing the level of serum cholesterol. From this work they concluded that the type of fat in the diet was more important than the amount of fat, which is consistent with earlier reports by Bragdon et al (1957) and Barnes et al (1959a). Greer et al (1966) also

reported that no significant effects on serum cholesterol levels were observed with pigs fed two protein levels (12% versus 18%) in a 15% fat diet. However low protein diets resulted in a greater incidence of lesions in the thoracic and abdominal aortas. The addition of 1% cholesterol to the diet resulted in significantly higher serum cholesterol levels and a higher incidence of lesions in the aortas and coronary arteries than observed with pigs fed the same diets without added cholesterol. This is not in agreement with previous results of Mattson et al (1972) who indicated that dietary cholesterol was the most important factor in influencing serum cholesterol levels. Greer et al (1966) also found that feeding tallow plus cholesterol resulted in higher serum cholesterol levels than feeding soybean oil plus cholesterol. These results are in agreement with earlier reports of Downie et al (1963) and Moreland et al (1963), but are in disagreement with the work of Reiser et al (1959) who reported that unsaturated fats in the diet increased the absorption of cholesterol whereas saturated fats depressed the absorption of cholesterol.

Hutagalung et al (1969) demonstrated that the addition of 1% cholesterol to pig diets resulted in a trend toward increased cholesterol concentrations in muscle and body fat and markedly elevated the cholesterol levels in liver and serum. The addition of 1% cholesterol to diets containing 12.5% lard resulted in significantly higher serum cholesterol than in diets without added lard or cholesterol.

Gupta et al (1974) reported that pigs fed a low protein (5%)-high fat (25%) diet supplemented with cholesterol (6 g/animal/day) had more extensive and severe lesions and more elevated serum cholesterol levels than animals fed a high protein (25%)-high fat (25%) diet supplemented with cholesterol (6 g/animal/day). They concluded that adequate levels of protein appear to have a protective effect on the occurrence of atherosclerotic lesions when animals are fed extremely high fat diets. Barnes et al (1959a) reported that serum cholesterol levels were not influenced by protein level. However, these same authors Barnes et al, (1959b) and Baker et al (1968) reported that serum cholesterol levels of pigs were elevated as dietary protein levels decreased, especially with extremely low protein levels.

Several additional studies have shown that adding fat (Hill, et al, 1971a, 1971b; Brooks et al, 1972) and cholesterol (Luginbuhl et al, 1969; Hill, 1972; Marsh et al, 1972; Nam et al, 1973; Lee et al, 1974) to swine diets increased blood cholesterol levels and increased the incidence of lesions in the arterial system. Yet, not all the results from adding fat have shown a positive correlation between the two. Barnes et al (1961) and Gresham et al (1964) have reported that the type of fat in the diet did not influence the level of serum cholesterol or the degree of lesion development. Barnes et al (1961) observed similar serum cholesterol levels in sows fed either hydrogenated fats or natural plant-fat mixtures. Link et al (1972) and Calvert

and Scott (1974) achieved only moderate hypercholesterolemia in female swine fed high fat diets and even then considerable fluctuations in cholesterol levels were observed. An attempt by Link et al (1972) to induce hypercholesterolemia was largely unsuccessful in male pigs.

Extremely high levels of serum cholesterol (890 mg/100 ml) have been observed in minature pigs fed a combination of 20% tallow, 3% cholesterol and 1% hog gall extract (Hill et al, 1975). In contrast the control animals in the experiment had mean serum cholesterol values of 92 mg/100 ml.

ii. Studies with Other Animals:

The effects of dietary fat or cholesterol on serum cholesterol levels and the development of atherosclerotic lesions has been studied with species other than swine. Increased levels of circulating cholesterol have been shown when fat or cholesterol was added to the diets of calves (Wiggers et al, 1971, 1973; Jacobson et al, 1974), rabbits (Ho et al, 1974; Borgman and Wardlaw, 1975), monkeys (Prathap, 1975), and chickens (Kruski and Narayan, 1972; Sklan, et al, 1974). However, Connor et al. (1967) reported that rabbits (a species which is highly susceptible to hypercholesterolemia) fed for one year on diets high in animal or vegetable fats produced no hypercholesterolemia and only slight atherosclerosis was noted in 50% of the rabbits fed the animal fat. When small amounts of cholesterol (0.25%) were added to the diet large

increases in serum cholesterol levels and considerable atherosclerosis resulted.

4. Cholesterol Values in Pigs

The reported blood cholesterol concentrations from a number of studies are shown in Table 2. The blood cholesterol levels of swine under normal dietary conditions may range from 80 to 190 mg/100 ml and were influenced by age, sex, breed, body weight, sampling time during the day as well as many other factors. Rothschild and Chapman (1976) and Rothschild et al (1975) reported that body weight and dam (heritability) could significantly influence serum cholesterol levels. Aherne et al (1976) also found blood cholesterol levels to increase with an increase in live-weight of control pigs.

Time of collection of the blood samples has been reported to influence the cholesterol values obtained. Tumbleson et al (1972) showed a 7% variation in the mean values of minature boars bled over an 11 day period when blood collections were made at midnight (66.8 mg/100 ml), 6 a.m. (63.0 mg/100 ml), noon (69.7 mg/100 ml) and 6 p.m. (70.5 mg/100 ml). Kellogg et al (1977) observed significant differences in tissue cholesterol between swine of different genetic backgrounds. They detected differences in cholesterol content of liver and muscle of Yorkshire, Hampshire, Duroc and Hampshire-Yorkshire pigs.

TABLE 2

SUMMARY: BLOOD CHOLESTEROL LEVELS IN SWINE

Treatment	No. Animals	Weight or Age	Total Cholesterol mg/100 ml	References
Fat Free Diet (72 days)	4	Weanling	125.5	Witz and Beeson, 1951
5% Fat (72 days)	2		130	
Normal	20	8.2-30 kg	88	Link, 1953a
Normal	11	56.8 kg	92	Rowsell et al, 1958
Butter	11	3-4 months	87	
Margarine	11		86	
Control Start	11		111	Rowsell et al, 1960
after 52 weeks	4	3-15 months	102	
Butter Start	11		107	
after 52 weeks	4		124	
Egg Yolk Start	11		117	
after 52 weeks	4		321	
15% Soybean oil	12	194 days	112	Greer et al, 1966
15% Soybean oil, 1% cholesterol	12		153	
15% Tallow	12		114	
15% Tallow, 1% cholesterol	12		184	
Normal		Minature pigs	70-90	Hill, 1966
High tallow			100+	
Normal		Weanling	190	McClellan et al, 1966
Normal		After weaning	80-100	
Control	10		96	Hutagalung et al, 1969
5% Corn oil	10	56.4 kg	90	
5% Lard	10		106	
5% Beef tallow	10		136	
5% Sheep tallow	10		130	
Low Fat		Mature	35.6-53.7	Kaneko and Cornelius, 1970
Rapeseed/Soybean meals	49	Growing	110-163	Bowland, 1975
Rapeseed meal/ Fababeans	32	Growing	121-148	Sarwar and Bowland, 1976
15% Rapeseed oil	64	4-5 weeks	122-154	Aherne et al, 1976
No added oil, 4 weeks	16		107	
16 weeks	16		114	
23 weeks	16		128	
Normal		Mature	69-165	Mia, 1976

a. Age and Sex Effects

Dvorak (1967) reported that serum cholesterol levels in piglets on the day of birth were 68 ± 18 mg/100 ml but had risen to 149 ± 32 mg/100 ml by 30 days of age. These and many other studies (Gyorkey and Reiser, 1964; Marsh et al, 1972) have confirmed that age is an important factor influencing the level of cholesterol found in the blood and that the incidence of atherosclerotic lesions is associated with high blood cholesterol levels. However, there are also studies that have reported no increase in cholesterol levels with age of swine (Gupta et al, 1974; Link et al, 1972). Tumbleson and Hutcheson (1976) suggest that serum cholesterol levels in pigs decrease with an increase in age, with females having higher mean values than males. At one month of age cholesterol values for males and females were 158 and 182 mg/100 ml, respectively whereas at 36 months of age values were 50 and 78 mg/100 ml for males and females respectively. This work was in agreement with earlier reports by Fillios et al (1958) who reported that under normal circumstances cholesterol levels are higher in females than males.

Link et al (1972) failed to produce hypercholesterolemia in male pigs but were able to do so in female pigs. Cox and Hale (1960) reported the effects of dietary hormones and fat level on serum cholesterol levels of swine. Their study involved 30 barrows fed two levels of testosterone

or a single level of stilbesterol (synthetic estrogenic compound) in diets supplemented with 5% or 10% beef tallow. The results showed that testosterone caused a marked reduction in serum cholesterol but there was no such effect with stilbesterol. From their results they suggested that castration contributed to hypercholesterolemia since boar pigs sampled had significantly lower serum cholesterol than barrows and essentially the same levels as those fed testosterone.

Review of research data with human subjects showed that higher levels of circulating cholesterol and increased atherosclerotic lesion formation occurred in males, with levels increasing with age in both men and women (Brusis and McGandy, 1971). Considerable variation in this study in cholesterol levels between the sexes and between different age groups within the same sex were observed and establishment of what normal cholesterol levels are is still not clearly resolved.

5. Influence of Dietary Fat or Oil on Pig Performance

a. Growth Rate and Feed Intake

A review of the data concerned with fat supplementation of pig diets showed that 10% added fat leads to a 10% reduction in feed intake, a 13% improvement in feed conversion efficiency and a 3 to 4% increase in growth rate (Agriculture Research Council, 1967). Since that review,

several other experiments have also shown that the level of dietary fat has a definite effect on pig performance.

Hutagalung et al (1969) showed that the addition of animal fats (lard, beef tallow or sheep tallow) to swine diets improved average daily gains and feed to gain ratios. Waterman et al (1973) studied the effects of low levels of supplemental tallow in finishing rations on pig performance. Three percent tallow in the diet reduced the time taken to reach market weight by 4 days and also reduced the metabolizable energy consumed per unit of gain by about 4%. Brooks (1972) and Allee et al (1970) reported that the addition of 10 to 20% fat in a pigs diet increased rate of gain. Brooks (1972) reported a negative correlation between feed/gain and energy concentration in the diet which is in agreement with the observations of Allee et al (1970) that the addition of 13% fat to a pig's diet improved the feed/gain ratio. The addition of vegetable oils to the diets of pigs allowed pig performance similar to that obtained from the addition of animal fats (Brooks et al, 1972). Friend and his coworkers in 1975 reported that growing-finishing pigs fed rapeseed oil or soybean oil diets (10 to 20% of the diet) had improved feed efficiency compared to pigs fed control diets but when a level of 20% of both oils were fed, body weight gain was significantly reduced ($P < 0.05$) for the first 4 weeks of the experiment. Aherne et al (1975, 1976) observed no significant differences in the average daily gain of growing pigs fed diets containing 15%

oil (rapeseed or soybean) or a control diet. Aherne et al (1976) reported that the inclusion of 15% rapeseed oil in the diet of pigs reduced the amount of feed required per kilogram liveweight by 20%. The improvement in feed conversion efficiency was similar to the values reported for other fats and oils (Agricultural Research Council, 1967).

According to the Agricultural Research Council (1967) at lower crude protein levels added fat to the diets of pigs caused a reduction in daily feed intake and little change in rate of gains. At a higher crude protein level added fat gave faster gains with little change in feed intake. Allee and Hines (1972) found that daily gains were not significantly affected by fat level when a constant calorie:protein ratio was maintained. With diets unadjusted for calorie:protein ratios daily gains decreased and metabolizable energy required per unit of gain increased when increasing amounts of fat were added to the diet of young pigs.

b. Digestibility of Fat or Oil in the Diet

Howard et al (1965) reported that the apparent digestibility of fat (ether extract) in diets of pigs was 44% for a 10% beef tallow diet, 79% for 10% maize oil diet and 82% for a standard commercial diet. In contrast the Agricultural Research Council (1967) cites the digestibility coefficients for pigs of a hydrolyzed fat mixture or beef tallow added as 5% of the diet were 76% and 86% and that of stabilized white greese added as 10% to 20% of the diet was 87% to 90%. The Agricultural Research Council also concluded that fat digestibility was affected by age and by chain length of the fatty acids in the fat.

Bayley and Lewis (1965) reported that the digestibilities of the individual fatty acids by the pig follow a similar pattern to that in other species, in that the unsaturated fatty acids are more easily absorbed than the saturated fatty acids. However, the absorption of a particular fatty acid is to a large extent influenced by the other fatty acids in the fat mixture which is fed. The wide variation in the reported values for the digestibility of beef tallow by pigs is unlikely to be due to this variation in the digestibility of the unsaturated fatty acids, since they are well digested (Bayley, 1976). Bayley suggested that the major source of variation in tallow digestibility was caused by the extent to which the saturated fatty acids (palmitic and stearic acids) are digested and that there may be a selective uptake of oleic acid by the intestinal mucosa.

6. The Influence of Dietary Volatile Fatty Acids on Pig Performance

a. Growth Rate and Feed Intake

Bowland, Young and Milligan (1971) reported that a volatile fatty acid mixture of 40% acetic, 40% propionic and 20% butyric could be added at levels of 2 and 8% in the diets of growing pigs without significantly influencing feed intake or rate of gain. When the volatile fatty acid mixture was fed at levels of 12% of the diet a significant

depression in daily gain and feed intake occurred. Cole et al (1975) reported that propionic acid (0.8%) treated barley was utilized as efficiently by pigs as was untreated dried barley (14% moisture).

The volatile fatty acid contribution from alimentary tract sources under normal dietary conditions could be equivalent to 15 to 28% of the daily maintenance energy requirements (184 to 330 Kcal) of the pig (Friend et al, 1964).

7. Propionate Metabolism

a. The Effect of Propionate on Ketogenesis

As early as 1933, Quastel and Wheatley demonstrated the effects of propionic acid on the oxidation of butyric acid in guinea pig liver homogenates. Quastel and Wheatley showed that when propionate, which contains an odd number of carbon atoms, was added to the butyrate incubations there was substantially less ketone bodies (acetoacetate, acetone, β-hydroxybutyrate) released. The addition of propionic acid and butyric acid at an equivalent concentration (0.17 M) reduced the formation of acetoacetic acid by liver homogenates by 84 percent. They suggested that the inhibitory effect of propionic acid may be due to a simple competition with butyric acid for the active surfaces involved in fatty acid oxidation. Studies with cows fed sodium propionate (Shultz, 1958) and with sheep given an intravenous

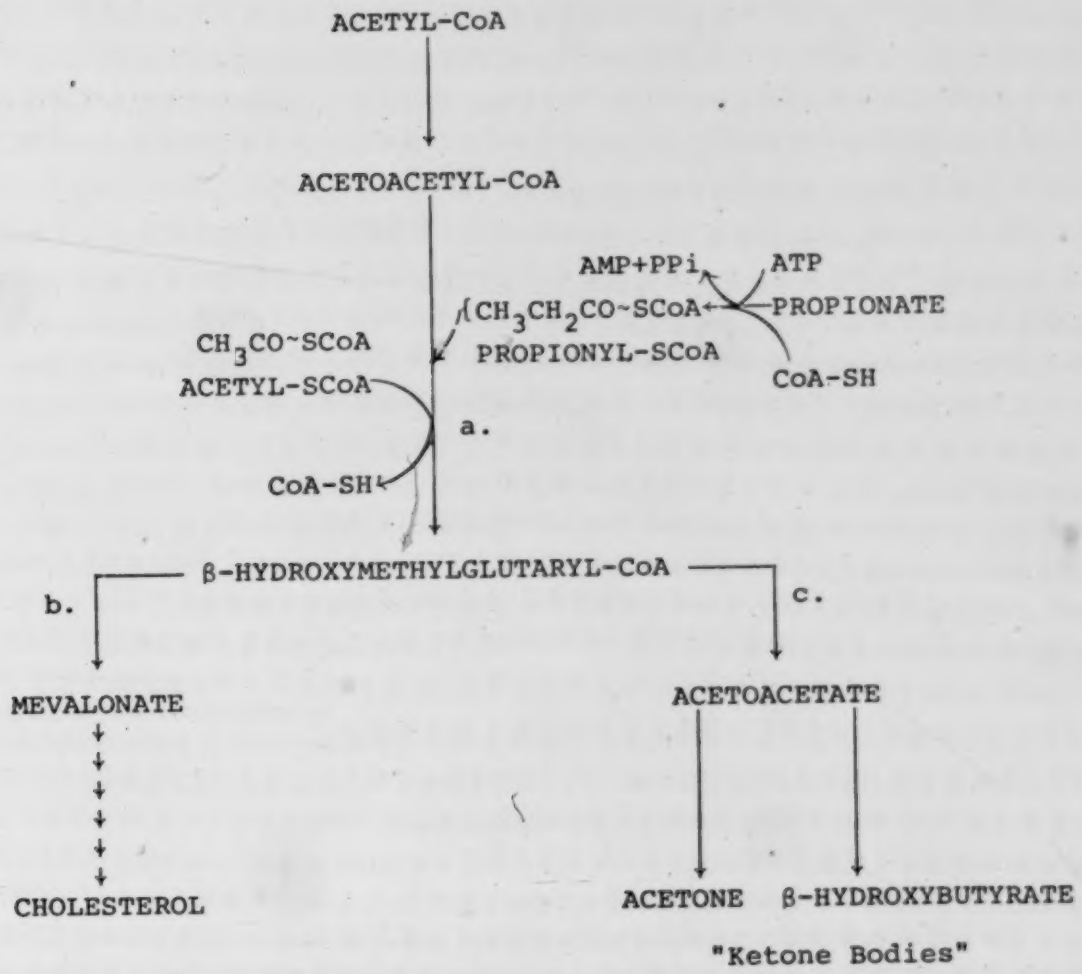
injection of sodium propionate (Reid and Mills, 1961) both have demonstrated the "antiketogenic" effect of propionate with reduced blood ketone levels. Subsequently Bush et al (1970) reported an inhibition of ketone body formation of 87% when bovine liver slices were incubated with butyrate plus propionate (9.0 µg/50 mg dry tissue) compared to incubations with butyrate alone (70.2 µg/50 mg dry tissue).

b. The Effect of Propionate on Cholesterolgenesis

The formation of cholesterol and of the three ketone bodies in liver tissue occurs through a common pathway (see Figure 1). The common pathway of formation of beta-hydroxymethylglutaryl-CoA (β-HMG-CoA) from acetoacetyl-CoA is catalyzed by the condensing enzyme beta-hydroxymethyl-glutaryl-CoA synthase (a.). From β-HMG-CoA, ketone bodies may be formed utilizing the enzyme β-HMG-CoA lyase (c.), or the formation of cholesterol can occur through the intermediate production of mevalonate in the presence of the enzyme β-HMG-CoA reductase (b.). Bush (1970) and Bush and Milligan (1971) suggested that the reduction of ketone body formation could be achieved by inhibition of the enzyme β-HMG-CoA synthase. In bovine liver incubations concentrations of 30 and 15 mM propionate inhibited β-HMG-CoA synthase by 58 and 30% respectively while propionyl-CoA at the same concentration as acetyl-CoA (0.5 mM) reduced the activity of the enzyme by 46%. Bush and Milligan concluded that propionyl-CoA, and propionate at high

FIGURE 1

SCHEMATIC REPRESENTATION OF CHOLESTEROL AND KETONE BODY FORMATION IN LIVER



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[a] β-Hydroxymethylglutaryl-CoA synthase.

[b] β-Hydroxymethylglutaryl-CoA reductase.

[c] β-Hydroxymethylglutaryl-CoA lyase.

concentrations inhibited the conversion of acetoacetyl-CoA to β-HMG-CoA. They suggested that *in vivo* propionyl-CoA rather than propionate would likely be involved in the inhibitions.

Propionyl-CoA has only one route of metabolism, that is to methylmalonyl-CoA and then to succinyl-CoA (Kaziro and Ochoa, 1964). This may favour its accumulation in the liver tissues at least relative to acetyl-CoA. Middleton (1967) and Higgins *et al* (1972) suggested that propionyl-CoA (and higher homologues) is a competitive inhibitor with respect to acetyl-CoA. From these suggestions and from the work of Bush (1970) one could speculate that propionyl-CoA may act competitively with acetyl-CoA for the active site on the β-HMG-CoA synthase enzyme molecule. The liver possesses high levels of β-HMG-CoA synthase activity and is a major site of cholesterol and ketone body formation (McGarry and Foster, 1969). Since the formation of cholesterol and ketones share a common pathway, one might assume that the inhibition of cholesterol formation could be achieved in the same manner as the inhibition of ketone body formation.

CONDITIONS COMMON TO ALL EXPERIMENTS

1. General Objectives: The experiments described below were designed to examine the effects of the dietary inclusion of propionic acid on plasma and tissue cholesterol levels of swine fed diets with or without the addition of high levels of fats or oils.

2. Experimental Procedures

a. Animals and diets: All experiments were carried out at the Edmonton Research Station from September 1975 to December 1976. All pigs used were crossbreds and of a similar age within each experiment. Barn temperature was maintained between 21-22°C and wood shavings or straw were usually provided as partial bedding. Feed was provided ad lib, except in the digestion trials, and pigs were always allowed free access to water. With the exception of Experiment 3, diets containing either 10% or 15% added tallow or rapeseed oil contained 15% more crude protein, minerals and vitamins than the non-fat diets to compensate for an expected decreased feed intake.

b. Collection of blood samples: Pigs in all trials were bled by anterior vena cava puncture (Carle and Dewhirst, 1942). Approximately 15 ml of blood was withdrawn at each bleeding. For plasma collection heparinized needles and syringes were used and the blood was then placed into 15 ml

tubes containing approximately one drop of heparin (1000 units/c.c.). Blood samples were then centrifuged at 2500 rpm (1.1xG) and the plasma transferred into 20 ml plastic or glass disposable screw-cap vials. Samples were stored at -30°C until analyzed.

STATISTICAL ANALYSIS

The experimental data (unless otherwise indicated) were analyzed using analyses of variance. Analyses of variance involving equal numbers of observations were analyzed according to the procedures given by Steel and Torrie (1960). In the case of Experiment 1, where one pig died, missing values were substituted from an equivalent pig of the same sex and in the same pen. Multiple comparison of means were made at the 5% ($P<0.05$) level of probability using Duncan's Multiple Range Test (Steel and Torrie, 1960).

The following symbols were used to denote tests of significance:

Symbol	Meaning
*	Means are significantly different at $P < 0.05$
**	Means are significantly different at $P < 0.01$
***	Means are significantly different at $P < 0.001$
a,b,c,d,e	Means bearing the same letter or no letters are not significantly different at $P < 0.05$
SEM	Standard error of the means
NS	Means are not significantly different at $P < 0.05$

METHODS OF CHEMICAL ANALYSIS

1. Feed and Feces Samples: Duplicate samples of feed were ground and analyzed for crude protein (N x 6.25), dry matter, ash and ether extract by A.O.A.C. (1975) methods. Fecal nitrogen was determined using the same method. A commercial "kel-pak"[5] was used as a catalyst in the Kjeldahl analyses, the ammonia was collected in 50 ml aliquots of 4% boric acid and titrated with standard H_2SO_4. Gross energy of feed and feces were determined in a Parr Oxygen Bomb Calorimeter[6] equipped with a Brown Electronik Recorder.[7]

2. Plasma Total Cholesterol: Plasma total cholesterol concentration was determined by the method described by Block, Jarrett and Levine (1966). Samples of plasma were thawed and duplicate samples of 0.5 ml pipetted into 20 x 150 mm screw-cap culture tubes. To each tube 9.5 ml of reagent grade isopropanol was added and the tubes were vigorously mixed for at least 45 seconds. The tubes were allowed to stand for 10 minutes and then centrifuged at 1000 rpm for 5 minutes. A 2 ml pasteur pipette was used to transfer an aliquot of the mixture into 2 ml autoanalyzer

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[5] Kel-pak; Matheson Scientific, East Rutherford New Jersey. Mixed catalyst supplies HgO, K_2SO_4 and $CuSO_4$.

[6] Parr Instrument Co., Moline, Illinois.

[7] Minneapolis-Honeywell Regulator Co., Philadelphia, Pennsylvania.

cups. Total plasma cholesterol was determined using a Technicon Autoanalyzer (Methodology N-24a).[8] The samples were run at a rate of 40 per hour. Absorbance was read at 550 nm in a 15 mm tubular flow cell. A $FeCl_3$[9] mixture was used as the color reagent. Sample values were compared directly to a standard cholesterol[10] curve.

3. Tissue Total Cholesterol: Samples of frozen liver, kidney, fat and muscle were freeze-dried[11] to a constant weight for 72 hours at a shelf temperature of 38°C. Large quantities of liver and kidney were finely ground using a Micro Analytical Mill[12] (no. 1) and put into 100 ml plastic capped bottles for analysis. Core samples of muscle and fat were put separately into 20 ml glass scintillation vials, freeze-dried, finely chopped using a small scapula, capped and kept at -30°C until analyzed. Analyses of tissue samples were by the method described by Naito and Lewis (1975). Approximately 150 mg duplicate samples of each tissue were placed in a 25 x 150 mm Teflon screw-cap culture tube. The sample was homogenized in 10 ml of reagent grade

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[8] Technicon Instruments Corp., Tarrytown, New York, U.S.A.

[9] Ferric Chloride (98%) J.T. Baker Chemical Comp., Phillipsburg, N.J., U.S.A. (825 mg $FeCl_3$ in 2000 ml concentrated glacial acetic acid and 1000 ml concentrate H_2SO_4).

[10] Cholesterol. Sigma Chemical Company, St. Louis, Mo., U.S.A.

[11] Repp Sublimator, Model SRC 42, Division of Virtis Co., Inc., Gardiner, New York, U.S.A.

[12] Canadian Laboratory Supplies (Canlab).

isopropanol using a Polytron Homogenizer[13] fitted with a size PT 10 ST generator. The mixture was allowed to stand for 10 minutes and then centrifuged at 200 x g for 10 minutes. The supernatant fluid was poured into 20 ml capped glass vials. Analysis for total cholesterol concentration was accomplished using the Autoanalyzer as previously described for plasma samples.

4. Determination of Cholesterol in Bile: The extraction and estimation of total cholesterol in bile samples was accomplished as described in Appendix A. Duplicate samples of bile were determined and the extract analyzed on the Autoanalyzer as previously described for plasma samples.

5. Estimation of Total Plasma Lipids: Analysis for total lipid per ml of plasma was determined as shown in Appendix B. Extraction of samples was made as outlined and extracts ran on a gas chromatograph[14] using a C_{17} internal standard[15]. Peak areas were measured using an integrator[16] attached to a recorder[17]. Identification of peaks were made with reference to standard peaks previously recorded.

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[13] Polytron Homogenizer PT10-35, Brinkman Instruments, Kinematica, Luzerne, Switzerland.

[14] Bendix Series 2500. Bendix International Operations, New York, N.Y., U.S.A.

[15] Heptadecanoic acid Lot #0528, Applied Science Laboratories Inc., Box 400, State College, PA.

[16] Autolab Minigrator, Technical Marketing Associates, Toronto, Canada.

[17] Fisher Recordall, Series 5000.

6. Determination of Fatty Acid Patterns of Tallow and Rapeseed Oil Samples: Fatty acid patterns of two tallow and one rapeseed oil sample were analyzed to determine the relative contents of predominant fatty acids for each fat or oil. The procedure describing the determinations are outlined in Appendix C. Identification of individual fatty acids was made using standard peaks previously determined. Samples were run on gas chromatograph as described previously for total lipids.

7. Determination of 7-dehydrocholesterol: Plasma samples collected for determination of 7-dehydrocholesterol concentrations were analyzed according to the method of Horton et al (1971). Some modifications such as increased volume size and the use of a second extraction are shown in the method outlined in Appendix D. Analysis of 7-dehydrocholesterol was measured as "fast-acting sterols" (Moore and Baumann, 1952) using the stable Liebermann-Burchard reagent described by Kim and Goldberg (1969). The absorbance at 625 nm was recorded using an ultraviolet Spectrophotometer[18]. Estimation of samples were derived from a curve obtained from standard 7-dehydrocholesterol[19] absorbances recorded at various concentrations.

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[18] SP 1800 Ultraviolet Spectrophotometer, Pye Unicam Ltd., Cambridge, England.

[19] Aldrich Chemical Comapny, Milwaukee, Wisc., U.S.A.. Inhibited with 15 - 20% methanol.

EXPERIMENT 1

1. Objective: The objective of Experiment 1 was to study the effects of the dietary inclusion of propionic acid on blood plasma and tissue cholesterol levels and on the performance of growing pigs fed diets of different fat content.

2. Experimental Procedures

a. Animals and diets: Sixty-four pigs of an average weight of 24 kg were randomly allotted to four replications of four dietary treatments. The four diets, as shown in Table 3, consisted of a control (C) diet or a diet containing 10% tallow (T), both fed with or without the addition of 5% propionic acid (PA). The diets were mixed at intervals of 2.5 to 3 weeks to avoid the possibility of development of rancidity in the feed. Each replication consisted of four pens with four pigs (2 barrows and 2 gilts) per pen. Animals were housed in approximately 1.2 x 3 meter pens on solid concrete floors. Pigs were weighed initially and every 2 weeks thereafter. Feed consumption on a pen basis was recorded every 2 weeks on the day on which pigs were weighed.

b. Collection of samples:

i. Blood samples: Blood samples were obtained from pigs at the start of the experiment and at the same time on days 7, 14, 21, 28, 49 and 70 of the experiment. Plasma was prepared as described previously.

TABLE 3

FORMULATION AND COMPOSITION OF DIETS

EXPERIMENT 1

DIETS	C	5%PA	10%T	10%T + 5%PA
Ingredients (percent)				
Barley	66.0	61.4	49.8	44.8
Wheat	17.3	15.3	13.0	11.4
Soybean meal (48.5 percent)	13.2	14.8	23.0	24.6
Stabilized tallow	--	--	10.0	10.0
Propionic acid[1]	--	5.0	--	5.0
Iodized salt	0.5	0.5	0.6	0.6
Ground limestone	1.0	1.0	1.2	1.2
Dicalcium phosphate	1.0	1.0	1.2	1.2
Vitamin-mineral premix[2]	1.0	1.0	1.2	1.2
Composition (calculated)				
Crude protein (percent)	16.0	16.0	18.4	18.4
Digestible energy (Kcal/kg)	3212	3308	3757	3855
Calcium (percent)	0.72	0.75	0.87	0.87
Phosphorous (percent)	0.56	0.55	0.59	0.58
Composition (analyzed)				
Crude protein (percent)	16.6	16.9	18.7	19.0
Crude fat (percent)	1.56	1.60	10.41	11.23
Gross energy (Kcal/kg)	3983	4068	4416	4584
Dry matter (percent)	90.9	91.2	91.9	91.8
Ash (percent)	5.8	5.0	5.7	5.9

[1] Propionic acid - 97.1% propionic, 2.9% acetic, less than 0.1% water. Celanese Canada Limited, Edmonton, Alberta, Canada.

[2] Supplied the following per kg of diet - 4400 I.U. Vitamin A; 665 I.U. Vitamin D_2; 11 I.U. Vitamin E; 66 mg Vitamin B_{12}; 11.1 mg riboflavin; 22.2 mg calcium pantothenate; 50 mg niacin; 55.7 mg choline chloride; 1.65 mg folic acid. It also provided cobalt 2.8 ppm; copper 24.6 ppm; iron 294.1 ppm; manganese 76.2 ppm; zinc 88.5 ppm and iodine 1.5 ppm. In addition it provided 110 mg oxytetracycline per kg of diet.

ii. Tissue samples: After 70 days on experiment pigs were slaughtered and tissue samples collected. A large portion of the median lobe of the liver and the entire right kidney were collected from each pig. Core samples of muscle and fat were also collected using a 27 cm diameter hand operated coring device. One back core (approximately 20 cm from the tail head and 5 cm 90° to the back line) and a leg core sample (approximately one-half the distance from the tail head to the first joint on the back of the leg) were collected. Muscle samples collected from the back and leg core were of the longissimus dorsi and semitendenosus respectively. Prior to the start of Experiment 1, tissue samples for comparative studies were also collected from eight pigs (4 barrows and 4 gilts) weighing approximately 33 kg (31 to 35 kg). All tissue samples were collected into sealed plastic bags and frozen at -30°C until analyzed.

iii. Bile samples: Gall bladders were carefully removed from the lobe of the liver and the bile was drained into 100 ml plastic disposable capped vials and frozen at -30°C until analyzed.

3. Digestion Study: Digestion studies were conducted during the experimental period. Two replications of eight pigs were randomly assigned to the four dietary treatments previously described (Table 4). In each replication there were one male and one female pig per treatment. The pigs in replication one were assigned to their respective treatments at an average

initial weight of 47.7 kg whereas those in replication two averaged 52.1 kg at the time of allottment. During each trial, the selected pigs were placed in raised, solid walled, wire mesh floored digestion cages. The pigs were allowed a 3 day period in which to become accustomed to the cages. No collections were made during this period. On the morning of the fourth day the cages were cleaned and collection trays were installed beneath the cages. Total fecal collections were made daily for 4 consecutive days after which pigs were removed and weighed.

During each of the digestion trials records and collection procedures were as follows. Pigs were fed at a level of 80% of the individual average daily feed consumption during the week prior to the trial. Pigs were fed three times daily and were allowed free access to water. Any feed that was not consumed during the trial was collected, dried at 60°C for 72 hours in a forced air oven[1] and allowed to stand at room temperature for several hours to arrive at an "air-dry" basis, before being weighed. Samples of feed were separately analyzed for each trial (see section on "Methods of Chemical Analysis").

Each morning during the collection period feces were collected and placed in a labelled plastic bag and stored at 3°C. After the fourth day of collection, total feces collections from each pig were thoroughly mixed, total weight was taken and approximately 600 g of wet feces were

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[1] Style 31, Despatch Oven Co., Minneapolis, Minn., U.S.A.

TABLE 4

COMPOSITION OF DIETS FOR DIGESTIBILITY TRIALS

EXPERIMENT 1

DIETS	C	5%PA	10%T	10%T + 5%PA
Composition (calculated)				
Crude protein (percent)	16.0	16.0	18.4	18.4
Digestible energy (Kcal/kg)	3212	3308	3757	3855
Calcium (percent)	0.72	0.72	0.87	0.87
Phosphorous (percent)	0.56	0.55	0.59	0.58
Digestibility Trial I				
Composition (analyzed)				
Crude protein (percent)	15.1	15.8	18.8	18.1
Crude fat (percent)	1.42	1.54	9.48	10.64
Gross energy (Kcal/kg)	3990	4059	4246	4580
Dry matter (percent)	89.1	90.6	90.8	91.4
Ash (percent)	4.9	4.2	5.8	5.5
Digestibility Trial II				
Composition (analyzed)				
Crude protein (percent)	16.2	14.6	18.4	18.2
Crude fat (percent)	1.52	1.38	10.52	13.99
Gross energy (Kcal/kg)	3932	3980	4470	4568
Dry matter (percent)	90.7	90.2	91.1	90.6
Ash (percent)	5.8	4.5	5.8	6.0

withdrawn and placed in an aluminum tray. The samples of feces were dried in a forced-air oven at 60°C for 72 hours, removed and allowed to equilabrate to an "air-dry" basis for several hours and then weighed for determination of air-dry weight. Each sample was individually ground in a (Size 8C and N) Laboratory Mill[2] with a 2 mm mesh screen.

Results and Discussion

1. Pig Performance; average daily gain (ADG) average daily feed intake (ADF) and efficiency of feed conversion (EFC):

The effects of the four dietary treatments on ADG, ADF and EFC are presented in Table 5. Statistically significant differences were noted for the above three parameters in all the time periods measured. As expected the inclusion of tallow in the diet decreased ($P < 0.001$) the overall (0 to 10 weeks) feed consumption by 16%. The inclusion of tallow also improved ($P < 0.001$) EFC by 18% and improved ADG by 3 to 4%. These results are consistent with the statement by the Agricultural Research Council (1967) that a 10% reduction in ADF, a 3 to 4% increase in ADG and a 13% improvement in feed conversion efficiency results when 10% tallow was added to the diet. These results are also in agreement with those obtained by Aherne et al (1976) who

[2] Christy and Norris Ltd., Chelmsford, England.

TABLE 5

PERFORMANCE OF PIGS FROM 0 TO 10 WEEKS ON EXPERIMENT[1]

DIET	C	5%PA	10%T	10%T + 5%PA	S.E.M.	SIGNIFICANCE
Initial Weight (kg)	24.5	24.8	24.6	25.0		
Final Weight (kg)	84.7	80.0	86.9	82.7		
Week 0-2						
ADF (kg)	1.78^{a}	1.45^{b}	1.28^{b}	1.37^{b}	0.103	*
ADG (kg)	0.78^{a}	0.66^{b}	0.80^{a}	0.71^{ab}	0.034	*
EFC (kg feed/kg gain)	2.29^{a}	2.21^{ab}	1.60^{b}	1.92^{ab}	0.145	*
Week 2-4						
ADF (kg)	2.39^{a}	1.96^{b}	2.35^{a}	1.81^{b}	0.093	**
ADG (kg)	0.71	0.77	0.78	0.82	0.037	NS
EFC (kg feed/kg gain)	3.37^{a}	2.54^{b}	3.03^{a}	2.21^{b}	0.111	***
Week 4-6						
ADF (kg)	2.76^{a}	2.40^{b}	2.48^{b}	2.16^{c}	0.077	**
ADG (kg)	1.03^{a}	0.88^{b}	1.09^{a}	0.98^{ab}	0.043	*
EFC (kg feed/kg gain)	2.68^{a}	2.74^{a}	2.28^{b}	2.19^{b}	0.103	**
Week 6-8						
ADF (kg)	3.13^{a}	2.75^{b}	2.74^{b}	2.36^{c}	0.080	***
ADG (kg)	0.94^{ab}	0.85^{bc}	0.99^{a}	0.80^{c}	0.042	*
EFC (kg feed/kg gain)	3.33^{a}	3.25^{a}	2.78^{b}	2.95^{ab}	0.132	*
Week 8-10						
ADF (kg)	3.71^{a}	3.03^{ab}	2.80^{bc}	2.49^{c}	0.144	***
ADG (kg)	0.85	0.79	0.80	0.80	0.052	NS
EFC (kg feed/kg gain)	4.36^{a}	3.84^{ab}	3.51^{b}	3.10^{b}	0.260	*
Overall 0-10						
ADF (kg)	2.75^{a}	2.32^{b}	2.33^{b}	2.04^{c}	0.057	***
ADG (kg)	0.86^{ab}	0.79^{c}	0.89^{a}	0.82^{bc}	0.017	**
EFC (kg feed/kg gain)	3.20^{a}	2.94^{b}	2.62^{c}	2.47^{c}	0.051	***

[1] Each diet represents a mean of 16 pigs.

found no significant differences in the ADG of pigs fed 15% oil or a control diet. Over the 10 weeks of the experiment the inclusion of propionic acid in diets with or without the addition of tallow significantly decreased ADF and ADG, and improved EFC compared to C diets. These results are in contrast to those reported by Bowland, Young and Milligan (1971) who reported that feeding volatile fatty acid mixtures at 2 to 8% of the diet did not significantly influence feed intake or rate of gain.

2. Digestibility Studies: The digestibility coefficients obtained for nitrogen and energy of the four diets are shown in Table 6. The digestibility coefficients for energy (DE) and nitrogen (DN) determined by the total collection procedure were not significantly different between treatments, replications or sexes. Values for DE were consistent with those reported by Onaghise (1976). There was a larger variation in the DN values obtained than was observed for DE. The digestibility coefficients for nitrogen were not consistently lower than those obtained for energy, as has been previously reported by Onaghise (1976) and Okai (1974). The inclusion of tallow or propionic acid both appeared to increase the digestibility of nitrogen, but the difference was not significant.

3. Blood Studies: The average plasma cholesterol values for pigs from each treatment are shown in Table 7. Pigs

TABLE 6

NITROGEN AND ENERGY DIGESTIBILITY DATA[1] (%)

DIET	C	5%PA	10%T	10%T + 5%PA	S.E.M.	SIGNIFICANCE
% Digestible Energy	79.4	76.6	79.6	79.6	1.05	NS
% Digestible Nitrogen	76.6	79.2	81.6	81.8	1.60	NS

[1] Each value is the mean for 4 pigs.

assigned to the diets containing tallow tended to have lower but not significantly different plasma cholesterol levels at the commencement of the experiment. Significant differences in plasma cholesterol were noted in pigs fed the four diets for 1, 7 and 10 weeks of the experiment. One week after the start of the experiment the plasma cholesterol levels of the pigs fed the C and the PA diets were similar to their respective starting levels, whereas the average cholesterol level of pigs fed the T diet had increased by 33 mg to 118 mg/100 ml which was significantly higher than that of pigs fed the other three diets. Pigs fed the T diet had the highest plasma cholesterol levels throughout the experiment but the differences were significant only in weeks 1 and 10. Animals fed the PA diet had the lowest ($P < 0.05$) average plasma cholesterol level in week 7 and week 10.

For each period sampled the cholesterol levels of pigs fed the T + PA diet were lower than those of pigs fed the T diet but the differences were significant only for weeks 1, 7 and 10. The addition of propionic acid to the C diet also tended to reduce plasma cholesterol levels but the differences were significant only in weeks 7 and 10 of the experiment.

The inclusion of tallow in the diet was not consistent in raising and maintaining plasma cholesterol levels in pigs as reported by Gupta *et al* (1974) and Greer *et al* (1966). Within each time period there were no significant differences in the plasma cholesterol levels of males

TABLE 7

PLASMA CHOLESTEROL CONCENTRATIONS FROM 0 TO 10 WEEKS ON EXPERIMENT[1]

DIET	C	5%PA	10%T	10%T + 5%PA	S.E.M.	SIGNIFICANCE
Plasma Cholesterol (mg/100 ml)						
Week 0	91	93	85	82	4.0	NS
Week 1	92[a]	91[a]	118[b]	97[a]	3.6	***
Week 2	96	92	106	99	4.9	NS
Week 3	89	88	99	94	4.0	NS
Week 4	86	84	96	93	6.1	NS
Week 7	88[ab]	72[c]	92[a]	83[b]	2.7	**
Week 10	87[a]	76[b]	98[c]	89[a]	1.5	***
Week 10 minus 0	- 4[a]	- 17[b]	+ 13[c]	+ 7[c]	3.8	--
Regression (b)	- 0.60	- 2.14*	- 0.56	- 0.49		
Standard error of b	0.34	0.42	1.31	0.81		

[1] Each diet represents a mean of duplicate values from 16 pigs.

or females fed the same diets. The changes in plasma cholesterol level with time observed with pigs fed the C diet suggests that plasma cholesterol does not increase with age in pigs fed low fat diets.

The differences in plasma cholesterol levels from start to finish (week 10 minus week 0) for each treatment group were compared against each other using a single degree of freedom comparison (Steel and Torrie, 1960). Results showed that the inclusion of tallow in the diet significantly increased plasma cholesterol level and that the inclusion of propionic acid in the control diet significantly decreased it compared to that of animals fed control diets. However a simple regression of plasma cholesterol on weeks for each diet (Table 7) indicated a significant decrease in plasma cholesterol for the propionic acid diet only ($b = -2.14$). It is worthy to note that the differences were tested at a statistical level of 0.05 and they would reach a higher significant level if a less stringent test were used.

4. Tissue and Bile Studies: The cholesterol concentrations of the tissue and bile samples of the experimental and pre-experimental pigs are shown in Table 8. No significant differences in cholesterol concentrations of any of the tissue or bile samples analyzed were observed between pre-experimental males and females. For comparisons of the pre-experimental samples with the 10 week samples of pigs fed the four diets, the means for the males and females of the

TABLE 8

TISSUE AND BILE CHOLESTEROL CONCENTRATIONS

(PRE- AND POST-TREATMENT)[1]

TREATMENT	C	5%PA	10%T	10%T + 5%PA	S.E.M.	SIGNIF.	PRE-TREATMENT	S.E.M. WITH PRE-TREATMENT
Tissue[2]								
Liver	414^{a}	385^{a}	420^{a}	426^{a}	15.0	NS	319^{b}	16.5
Kidney	393^{a}	350^{b}	392^{a}	353^{b}	9.5	**	386^{a}	10.4
Back Muscle	71^{a}	68^{a}	74^{a}	75^{a}	3.3	NS	88^{b}	3.6
Back Fat	175^{a}	214^{b}	191^{ab}	170^{a}	7.7	**	140^{c}	8.4
Leg Muscle	86	98	101	99	7.0	NS	104	7.7
Leg Fat	209^{ab}	269^{a}	216^{ab}	190^{ab}	22.3	NS	169^{b}	24.5
Bile[3]	167^{a}	158^{a}	180^{ab}	166^{a}	13.2	NS	224^{b}	14.5

[1] Post-treatment values are means of 16 pigs.
Pre-treatment values are means of 8 pigs.

[2] mg/100 g wet weight of tissue.

[3] mg/100 ml of bile.

pre-experimental and each of the four diets were combined. These means were tested using multiple range tests (Dunnett's procedure; Steel and Torrie, 1960) for the treatment and sex by treatment interactions. The measure of error variation was the "valid error" obtained from the ANOVA of the specific trait for the four diets.

Comparisons of pre-test values with pigs fed C diets showed that cholesterol levels in muscle and bile tended to decrease with age of the pig. In contrast fat, liver and kidney all showed increases in cholesterol content with age.

Statistical analysis of samples from animals fed the four diets showed that the addition of propionic acid to C diets decreased ($P < 0.05$) the cholesterol content of kidney and had no significant effect on the cholesterol levels of liver, muscle and bile. The addition of propionic acid alone increased the cholesterol level of both fat samples but only in the case of back fat samples was the increase significant. Relative to the control diet the inclusion of tallow in the diet resulted in a non-significant increase in the cholesterol content of the bile and in all other tissues tested except kidney in which the level remained unchanged.

Similar to the observations for the PA diet the addition of propionic acid to the T diet decreased ($P < 0.05$) the cholesterol content of the kidney, but had no significant effect on the cholesterol contents of any of the other

tissues or bile samples. It is interesting to note that the addition of propionic acid to the T diet decreased the cholesterol content of both back and leg fat samples which is in contrast to the effect of propionic acid addition to the C diet. No significant differences between barrows and gilts were observed except in back and leg fat samples where females had significantly higher cholesterol concentrations than males.

The levels of tissue and bile cholesterol as presented in Table 8 are in agreement with those of Marsh et al (1972) who reported a range of 57 to 64 mg/100 g for adipose tissue, 57 to 64 mg/100 g for muscle and 86 to 194 mg/100 ml for bile samples. Hutagalung et al (1969) observed that muscle, subcutaneous fat and liver samples from pigs fed control diets had cholesterol concentrations of 36, 105 and 302 mg/100 g of tissue, respectively. They also observed a significant increase in the cholesterol concentration of these tissues with 5% added dietary fat. Recently Kellogg et al (1977) observed significant differences in the tissue levels of different breeds of pigs. In this study the range in cholesterol levels were 479 to 542 mg/100g, 504 to 537 mg/100 g, and 109 to 139 mg/100 g for liver, back fat and muscle tissue respectively.

Summary

Sixty-four crossbred pigs, approximately 25 kg in weight were allotted to four diets - a control diet and a 10% tallow diet both with or without the addition of 5% propionic acid. The experiment was conducted for 10 weeks. Plasma cholesterol levels were determined on weeks 0, 1, 2, 3, 4, 7 and 10. Pig performance was recorded every 2 weeks. After 10 weeks the pigs were slaughtered and samples of liver, kidney, back muscle and fat, leg muscle and fat, and bile were collected for cholesterol analysis. Tissue and bile samples were also collected from 8 pre-treatment pigs. A digestibility study using the four diets was also performed. The results indicated:

1. Dietary propionic acid tended to depress plasma cholesterol levels in swine, while the addition of tallow to the diet tended to increase plasma cholesterol levels. No significant differences in plasma cholesterol was observed between males and females.

2. The inclusion of tallow in the diet increased the cholesterol content of bile and tissue samples. The inclusion of propionic acid significantly lowered kidney cholesterol content. No significant differences in liver, back muscle, leg muscle and fat, and bile cholesterol concentrations were observed for any of the four treatments.

3. The inclusion of propionic acid in the diet improved EFC, but ADG and ADF were slightly less than those

of pigs fed the control diet. Overall the presence of tallow improved ADG and EFC and decreased the ADF.

4. Digestibility coefficients for DE and DN were not significantly different between treatments.

EXPERIMENT 2

1. Objectives: The objective of this experiment was to study the effects on blood cholesterol concentrations (over 20 days) of including 15% tallow or 10% tallow plus 10% sugar in isonitrogenous diets of growing pigs.

2. Experimental Procedures

a. Animals and diets: Twelve pigs of an initial average weight of 27 kg were randomly allotted to three dietary treatments, control (C), 10% tallow plus 10% sugar (T + S) and a 15% tallow (T) (Table 9). The diets were formulated to contain 16% crude protein. The mineral and vitamin levels of the fat supplemented diets was increased by 15% to compensate for an expected reduced feed intake in diets containing added tallow. Four animals (2 barrows and 2 gilts) per treatment were penned in concrete floor pens measuring 1.4 m x 3.9 m.

Pig weights and feed consumption for each pen were recorded on day 0, 10 and 20 of the experimental period.

b. Collection of blood samples: Blood plasma was collected from each pig in the morning of days 0, 5, 10 and 20 of the experiment.

TABLE 9

FORMULATION AND COMPOSITION OF DIETS

EXPERIMENT 2

DIETS	C	10%T + 10%S	15%T
Ingredients (percent)			
Barley	66.0	44.9	50.2
Wheat	17.3	11.2	12.5
Soybean meal (48.5 percent)	13.2	19.7	18.1
Stabilized tallow	--	10.0	15.0
Sugar	--	10.0	--
Iodized salt	0.5	0.6	0.6
Ground limestone	1.0	1.2	1.2
Dicalcium phosphate	1.0	1.2	1.2
Vitamin-mineral premix[1]	1.0	1.2	1.2
Composition (calculated)			
Crude protein (percent)	16.0	16.0	16.0
Digestible energy (Kcal/kg)	3212	3790	3962
Calcium (percent)	0.72	0.85	0.85
Phosphorous (percent)	0.56	0.54	0.56
Composition (analyzed)			
Crude protein (percent)	16.9	16.2	16.2
Crude fat (percent)	1.32	10.36	12.98
Gross energy (Kcal/kg)	3884	4337	4601
Dry matter (percent)	89.9	90.9	90.0
Ash (percent)	5.4	5.2	4.6

[1] See Table 3

Results and Discussion

1. Pig Performance (ADG, ADF, and EFC): The average daily gains, average daily feed intakes and the efficiency of feed conversion are shown in Table 10. No statistical analyses were performed on ADF or EFC because feed intakes were recorded on a pen basis, with only one pen of pigs per treatment. No significant differences were observed in ADG for the three treatments tested. Although the results were not significant, pigs fed diets containing sugar consumed more feed and had higher ADG than pigs fed the other diets. The EFC of pigs fed the 10%T + 10%S or the 15%T diet were improved by 10 to 15% respectively compared to pigs fed the C diet. As expected the ADF of pigs fed the 15%T diet was 0.24 kg per day and 0.33 kg per day lower than C fed and 10%T + 10%S fed pigs respectively.

2. Blood Studies: The average plasma total cholesterol concentrations of the pigs fed the three diets are shown in Table 10. Statistically significant differences were observed between the three diets on days 0, 5 and 20 of the experiment. At the start of the experiment (Day 0), pigs allotted to the 15%T diet had lower ($P < 0.05$) plasma cholesterol levels than the other two diets. After 5 days on the experiment, pigs fed the C diet had significantly lower plasma cholesterol levels than pigs fed the other two diets. At 10 days, however, there were no significant differences in cholesterol

TABLE 10

PERFORMANCE AND PLASMA CHOLESTEROL CONCENTRATIONS OF PIGS[1]

TREATMENT	C	10%T + 10%S	15%T	S.E.M.	SIGNIFICANCE
Performance Data (0 to 20 days)					
Average initial wt. (kg)	27.2	28.0	25.8		
Average final wt. (kg)	43.4	46.9	42.1		
ADG (kg)	0.82	0.94	0.82	0.040	NS
ADF (kg)	1.99	2.08	1.75	--	--
EFC (kg feed/kg gain)	2.43	2.21	2.14	--	--
Plasma Cholesterol (mg/100 ml)					
Day 0	106^{a}	112^{a}	91^{b}	3.8	*
Day 5	99^{a}	119^{b}	112^{b}	3.9	*
Day 10	103	116	116	3.2	NS
Day 20	109^{a}	111^{a}	137^{b}	5.6	*
Day 20 minus Day 0	$+3^{a}$	-1^{a}	$+46^{b}$	5.9	--

[1] Each dietary treatment value represents a mean of 4 pigs

concentrations between pigs fed any of the three treatments. At the conclusion of the experiment (Day 20) pigs fed the 15%T diet had significantly higher (approximately 24% higher) cholesterol levels than pigs fed the other two diets. For the 20 day test period the addition of 15% tallow to the diet resulted in a highly significant increase in plasma cholesterol concentrations (46 mg/100 ml) while pigs fed the remaining diets showed very little change. No significant differences in plasma cholesterol of males or females were observed.

The increase in plasma cholesterol for pigs fed the 15%T diet is far greater than was observed at week 3 of Experiment 1 when pigs were fed a 10% tallow diet. Whether this increase in cholesterol in this experiment is a reflection of the higher tallow in the diet and/or the fact that the diets were not adjusted for calorie : protein ratio in this experiment is not known.

Summary

Crossbred pigs, weighing approximately 27 kg, were fed an isonitrogenous 16% grower ration containing either no tallow or sugar (C), 15% tallow (T), or 10% tallow plus 10% sugar (T + S). The experiment was conducted for 20 days. Pig performance and also plasma cholesterol levels at days 0, 5, 10 and 20 of the experiment demonstrated:

1. The inclusion of tallow plus sugar in pig diets did not have any significant influence on the level of plasma cholesterol. The inclusion of tallow alone significantly increased plasma cholesterol levels.

2. Diets containing 10%T + 10%S increased ADF and ADG and improved EFC. Diets containing 15%T reduced ADF and improved EFC without affecting ADG. These differences were not significant.

EXPERIMENT 3

1. Objectives:

a. The first objective was to observe the effects on plasma cholesterol concentrations of including tallow, rapeseed oil or cholesterol in diets of growing pigs for a period of 20 days.

b. The second objective was to determine the effects of sampling method, plasma fractionation, and the use of different or no anticoagulants on the cholesterol values of pigs.

2. Experimental Procedures

a. Animals and diets: Thirty pigs were randomly allotted by sex to five dietary treatments, with one pen of three gilts and one pen of three barrows per treatment. Pigs of an initial average weight of 23.6 kg were housed in pens (1.2 m x 3 m) on solid concrete floors. Pigs were fed a control (C) grower diet or a grower diet containing either 10% or 15% tallow (T), 15% rapeseed oil (RSO) or 1% cholesterol (CH) (Table 11). The previously described adjustments in protein, mineral and vitamins were made in the tallow and rapeseed oil supplemented diets.

Pigs were weighed at the start and completion of the experimental period and total feed consumption was recorded.

TABLE 11

FORMULATION AND COMPOSITION OF DIETS

EXPERIMENT 3

DIETS	C	10%T	15%T	15%RSO	1%CH
Ingredients (percent)					
Barley	66.0	49.8	44.9	44.9	66.1
Wheat	17.3	13.0	11.3	11.3	15.8
Soybean meal (48.5 percent)	13.2	23.0	24.6	24.6	13.6
Stabilized tallow	--	10.0	15.0	--	--
Rapeseed oil[1]	--	--	--	15.0	--
Cholesterol[2]	--	--	--	--	1.0
Iodized salt	0.5	0.6	0.6	0.6	0.5
Ground limestone	1.0	1.2	1.2	1.2	1.0
Dicalcium phosphate	1.0	1.2	1.2	1.2	1.0
Vitamin-mineral premix[3]	1.0	1.2	1.2	1.2	1.0
Composition (calculated)					
Crude protein (percent)	16.0	18.4	18.4	18.4	16.0
Digestible energy (Kcal/kg)	3212	3757	4014	4087	3177
Calcium (percent)	0.72	0.87	0.87	0.87	0.72
Phosphorous (percent)	0.56	0.59	0.58	0.58	0.56
Composition (analyzed)					
Crude protein (percent)	16.2	18.2	18.2	17.9	15.4
Crude fat (percent)	1.45	9.41	16.50	15.06	2.79
Gross energy (Kcal/kg)	3783	4398	4668	4531	3872
Dry matter (percent)	87.9	89.7	90.2	90.2	88.0
Ash (percent)	5.1	4.9	5.3	5.2	4.3

[1] Tower rapeseed oil.

[2] Nutritional Biochemical Corporation, Cleveland, Ohio, U.S.A.

[3] See Table 3.

b. Collection of samples: Blood plasma was collected from each pig in the morning of days 0, 5, 10 and 20 of the experiment.

On completion of part (a) of this experiment, six pigs from the control diet, and six pigs whose cholesterol levels were very high and six pigs with low plasma cholesterol levels were selected from the other treatment groups to determine the effects of different blood collection and sampling methods on cholesterol values. Six pigs (two pigs from each category described above) were randomly allotted to each of the three different sampling and collection procedures (A, B or C) as shown in Table 13. To determine the effects different anticoagulants have on cholesterol determinations (A) 10 ml of blood was collected using needles and tubes containing either heparin, EDTA[3], or no anticoagulant (serum). To determine the possible fractionation effects within heparinized plasma samples (B) two 10 ml plasma samples were withdrawn consecutively from each of six pigs. Cholesterol determinations were performed on the entire plasma sample or where upper and lower fractions of centrifuged plasma were required, the top and bottom halves of one of the plasma samples were pipetted into separate 20 ml capped glass vials. Due to sampling problems only three pigs were

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[3] Ethylenediaminetetraacetic acid, Fisher Scientific Company, Fairlawn, New Jersey, U.S.A.

sampled to determine the differences in cholesterol concentrations of arterial versus venous blood (C). Two consecutive 10 ml serum samples were withdrawn from each of 3 pigs for this study.

All plasma sampled was collected as described in Experiment 1. Serum samples were collected using clean syringes and tubes. Samples were allowed to stand for 10 minutes and then centrifuged at 2500 rpm to obtain the serum. All samples were stored at -30°C until analyzed.

Results and Discussion

1. Pig Performance (ADG, ADF and EFC): The average daily gain, average daily feed intake and efficiency of feed conversion of each of the five dietary treatments are shown in Table 12. Performance of pigs on all diets over the 20 day period was good and results were generally similar to those observed in Experiment 1. As expected the increase in the energy or fat content of the diet decreased daily feed intake, and improved both the ADG and EFC. Pigs fed the 15% RSO diets showed a higher ($P < 0.05$) ADG than pigs fed all other diets.

2. Blood Studies: The average plasma cholesterol concentrations for pigs fed each of the five diets are shown in Table 12. At the start of the trial (Day 0), no significant differences

TABLE 12

PERFORMANCE AND PLASMA CHOLESTEROL CONCENTRATIONS OF PIGS[1]

TREATMENT	C	10%T	15%T	15%RSO	1%CH	S.E.M.	SIGNIFICANCE
Performance Data (0 to 20 days)							
Average initial wt. (kg)	24.0	21.7	22.5	26.8	23.0		
Average final wt. (kg)	38.4	37.2	38.8	45.0	37.7		
ADG (kg)	0.72^{a}	0.76^{a}	0.82^{a}	0.92^{b}	0.74^{a}	0.032	***
ADF (kg)	1.85	1.67	1.55	1.64	1.82	0.095	NS
EFC (kg FEED/kg GAIN)	2.58^{a}	2.18^{bc}	1.90^{cd}	1.79^{d}	2.46^{ab}	0.088	*
Plasma Cholesterol (mg/100 ml)							
Day 0	$93_{}$	$91_{}$	$86_{}$	$100_{}$	$86_{}$	4.9	NS
Day 5	82^{a}	102^{bc}	104^{c}	110^{c}	87^{ab}	5.6	**
Day 10	86^{a}	102^{ab}	111^{bc}	121^{c}	93^{ab}	5.9	**
Day 20	86^{a}	102^{abc}	111^{bc}	122^{c}	98^{ab}	6.7	*
Day 20 minus Day 0	-7^{a}	$+11^{b}$	$+25^{b}$	$+22^{b}$	$+11^{b}$	4.8	--

[1] Each value represents a mean of 6 pigs.

were observed in the mean plasma cholesterol values of the pigs allotted to the five treatments. Pigs fed the 15%T or the RSO diets had significantly higher blood cholesterol levels on day 5, 10, and 20 of the experiment than those on the other treatments. There was no significant difference between the plasma cholesterol levels of pigs fed the 15%T or 15%RSO diets. Pigs fed the 10%T diets were only different ($P < 0.05$) from C fed pigs on Day 5 of the experiment. Plasma cholesterol of pigs fed diets containing 1% cholesterol (1%CH) did not significantly differ from those of C fed or 10%T fed pigs at any time of the bleeding periods. The addition of 1% cholesterol to the diet led to the same rise in plasma cholesterol as that obtained by the addition of 10%T. The observations from feeding 1% cholesterol in this experiment are in agreement with those of Hutagalung et al (1969) who reported that the addition of 1% cholesterol to the diet of pigs resulted in an elevation of serum cholesterol. Hutagalung et al found that even though dietary cholesterol alone elevated serum cholesterol levels, the addition of 12.5% lard and 1% cholesterol to the diet gave significantly greater serum cholesterol levels than the additive effects resulting from the additions of fat and cholesterol alone.

For the 20 day test period pigs fed either 15%T or 15%RSO showed an increase in plasma cholesterol levels which was twice that of pigs fed either 10%T or 1%CH. Control fed pigs had a net decrease in plasma cholesterol

of 7 mg/100 ml from Day 0 to Day 20. These results are in agreement with those of Greer et al (1966) who reported that pigs fed tallow had higher serum cholesterol levels than pigs fed soybean oil. No significant differences in plasma cholesterol were observed between male or female pigs at any time during the experimental period.

3. Blood Sampling and Collection Study: The cholesterol concentrations of plasma or serum collected and sampled by different methods are shown in Table 13. No significant differences were noted in any of the samples. The effect of anticoagulants (A), serum, or plasma collected with either heparin or EDTA as anticoagulants, gave very similar cholesterol values. These results were in agreement with Chen et al (1976) who reported that it was generally agreed that EDTA plasma or serum gave comparable results. There appeared to be no difference in the cholesterol level of plasma taken from the top and bottom layers of the sample (B). Cholesterol determinations were also similar in arterial or venous blood from the same pig (C). These results suggest that the methods of blood sampling and collection used in this study are unlikely to significantly influence the values of cholesterol determined.

TABLE 13

METHOD OF BLOOD SAMPLING ON BLOOD CHOLESTEROL CONCENTRATIONS (mg/100 ml)[1]

A. TREATMENT	HEPARIN PLASMA	EDTA PLASMA	SERUM	ANIMAL MEAN	S.E.M.	SIGNIFICANCE
Animal 1	89	80	79	82		
2	83	78	76	79		
3	150	143	145	146		
4	94	98	93	95		
5	98	96	98	97		
6	144	140	144	143		
Overall Mean	109.5	105.7	105.8		1.13	NS

B. TREATMENT	TOTAL PLASMA	TOP PLASMA	BOTTOM PLASMA			
Animal 7	114	112	114	113		
8	82	84	84	84		
9	126	126	124	125		
10	111	115	114	113		
11	102	106	114	108		
12	158	170	172	166		
Overall Mean	115.6	119.0	120.1		1.50	NS

C. TREATMENT	ARTERIAL SERUM	VENOUS SERUM				
Animal 13	82	84		83		
14	111	106		108		
15	153	151		152		
Overall Mean	115.2	113.5			1.43	NS

[1] Each value represents the mean of duplicate samples.

Summary

Thirty crossbred pigs, weighing approximately 24 kg were fed grower diets either containing either 10% tallow (T), 15% tallow (T), 15% rapeseed oil (RSO), or 1% cholesterol (CH). The experiment was conducted for 20 days. Overall pig performance as well as plasma cholesterol levels on days 0, 5, 10, and 20 were measured. Blood sampling and collection techniques were also tested. The results indicated:

1. The inclusion of 15% tallow or rapeseed oil significantly affected the level of plasma cholesterol, with the largest increases over 20 days observed with pigs fed 15%T. The increase in plasma cholesterol obtained with 10%T was about half that observed with feeding 15%T.

2. The effect of feeding 1%CH on plasma cholesterol levels was not significantly different from control pigs, with values observed equivalent to those of pigs fed 10%T.

3. As expected the addition of tallow or rapeseed oil to the diets reduced ADF, and improved ADG and EFC.

4. Blood sampling and collection techniques did not significantly influence the values of cholesterol determined.

EXPERIMENT 4

1. Objectives:

a. The primary objective of this experiment was to determine the effects of dietary propionic acid on blood cholesterol levels in hypercholesterolemic growing pigs.

b. A second objective was to determine the effects of a 12 hour starvation period on blood cholesterol values.

2. Experimental Procedures

a. Animals, diets, and blood collection: Ninety-five pigs of an average initial weight of 18 kg (range 13.6 to 22.7 kg) were allotted to 36 pens with a maximum of three pigs to a pen. The pigs were fed a 15% tallow (T) grower diet (Table 14) for a period of 10 days. Pen size was 0.6 m x 1.2 m with half-slatted concrete floors.

Plasma samples were collected from each of the 95 pigs at 8:30 A.M. on the tenth day of the feeding period. Plasma cholesterol was determined within 24 hours of the conclusion of the trial.

On the basis of the plasma cholesterol concentrations pigs were categorized as being of high (120 - 150 mg/100 ml) moderate (100 - 120 mg/100 ml) or low (less than 100 mg/100 ml) cholesterol levels. Twenty-seven pigs from the 120 to 150 mg/100 ml cholesterol group were randomly allotted to

TABLE 14

FORMULATION AND COMPOSITION OF DIETS

EXPERIMENT 4

DIETS	C	15%T	15%T + 5%PA
Ingredients (percent)			
Barley	66.0	44.9	40.0
Wheat	17.3	11.3	9.6
Soybean meal (48.5 percent)	13.2	24.6	26.2
Stabilized tallow	--	15.0	15.0
Propionic acid[1]	--	--	5.0
Iodized salt	0.5	0.5	0.6
Ground limestone	1.0	1.0	1.2
Dicalcium phosphate	1.0	1.2	1.2
Vitamin-mineral premix[2]	1.0	1.2	1.2
Composition (calculated)			
Crude protein (percent)	16.3	18.4	18.4
Digestible energy (Kcal/kg)	3212	4014	4111
Calcium (percent)	0.72	0.87	0.87
Phosphorous (percent)	0.56	0.58	0.56
Composition (analyzed)			
Crude protein (percent)	16.0	17.1	18.1
Crude fat (percent)	1.85	15.36	16.94
Gross energy (Kcal/kg)	3830	4612	4760
Dry matter (percent)	88.2	91.1	89.1
Ash (percent)	4.5	5.6	5.9

[1] Celanese Canada Ltd. (for composition see Table 3)

[2] The premix provided the following per killigram of diet: 120.0 mg Zinc; 10.0 mg Copper; 49.0 mg Manganese; 100.0 mg Iron; 45 IU Vitamin E; 7500 IU Vitamin A; 700 IU Vitamin D_3; 12 mg Riboflavin; 45 mg Niacin; 27 mg Calcium Pantothenate; 28 mcg Vitamin B_{12}; 500 mg Furizolidone.

the three dietary treatments shown in Table 14. Each treatment group consisted of 3 pens (0.6 x 1.2 m) of 3 pigs per pen. One treatment group was maintained on the 15% tallow (T) diet, whereas the other two treatments groups received either a control (C) diet or a 15% tallow plus 5% propionic acid (T + PA) supplemented diet. Animals were fed these diets for a period of 20 days. Plasma samples for cholesterol determinations were collected from each pig on the morning of days 0, 5, 10 and 20 while animals were maintained on these three diets.

To determine what effect total lipid levels in plasma may have on the values obtained for cholesterol in determinations of plasma total cholesterol, plasma samples from 12 pigs (4 per treatment) were randomly selected on both Day 0 and Day 20. An estimation of total lipids present in the plasma and its' relationship to total plasma cholesterol was determined.

After the initial characterization of pigs into high, moderate or low cholesterol groups, two pens of 3 pigs from each category of the remaining 95 pre-test pigs were starved for 12 hours and then bled. Plasma cholesterol concentrations of initial and 12 hours starved animals were compared.

Results and Discussion

1. Blood Studies: The results of the average plasma cholesterol concentrations of the pigs fed control (C), 15% tallow (T), 15% tallow plus 5% propionic acid (T + PA) diets are shown in Table 15.

All 27 pigs allotted to the three diets were pigs with the highest cholesterol levels (hypercholesterolemic pigs) selected from 95 pigs which had previously been fed a 15%T diet for 10 days. At the time of allottment to their respective diets there were no significant differences in the mean plasma cholesterol levels of the three treatment groups. Five and ten days after the pigs were put on test the C pigs had significantly lower plasma cholesterol levels (92 mg/100 ml) than either the 15%T or the T + PA fed pigs. No significant differences were observed between the treatments on Day 20, though the plasma cholesterol level of the 15%T diet was 26 mg/100 ml greater than that of the control pigs.

In viewing the overall effect from Day 0 to 20, C fed pigs decreased their cholesterol levels by 22 mg/100 ml, T fed pigs increased by 6 mg/100 ml and T + PA fed pigs showed no change. The change from a 15%T diet on Day 0 to the C diet reduced plasma cholesterol levels within 5 days from 129 mg to 92 mg/100 ml. Whether this reduction in plasma cholesterol reflects the reduction in the fat content of the diet or a reduced feed intake is not clear.

TABLE 15

PLASMA CHOLESTEROL CONCENTRATIONS OF PIGS ON EXPERIMENT[1]

TREATMENT	C	15%T	15%T + 5%PA	S.E.M.	SIGNIFICANCE
Plasma Cholesterol (mg/100 ml)					
Day 0	129	127	125	3.4	NS
Day 5	92[a]	129[b]	123[b]	5.4	**
Day 10	92[a]	126[b]	115[b]	3.6	**
Day 20	107	133	125	6.7	NS
Day 20 minus Day 0	- 22[a]	+ 6[b]	0[b]	5.3	--

[1] Each value represents a mean of 9 pigs.

The inclusion of 5% propionic acid with 15% tallow in the diet resulted in a slight decrease in plasma cholesterol levels, but values obtained were not significantly lower than those obtained with pigs fed the 15%T diet. The trends in plasma cholesterol values of pigs fed the propionic acid diet in this experiment were similar to those observed in Experiment 1. Using hypercholesterolemic type pigs at the onset of the trial resulted in a reduction in plasma cholesterol in pigs fed the C diet, but not with the other two diets. The lack of reduction of plasma cholesterol levels from feeding the T + PA diet may be a result of using hypercholesterolemic type pigs in the experiment.

2. Relationship of Plasma Cholesterol and Plasma Lipid Content: The plasma samples from 12 pigs (4 per treatment were randomly selected from plasma samples from both Day 0 and 20 of the above experiment to determine what effect total plasma lipid values may have on the values obtained for cholesterol in determinations of plasma total cholesterol. The total plasma cholesterol and estimated total plasma lipid from the 24 pairs of observations are shown in Table 16. A simple regression of plasma cholesterol on total lipid gave a value of $b = 0.104 \pm 0.033$. The square of the multiple correlation coefficient (r^2) equalled 0.32 which indicated that only 32% of the variation in values of lipid and cholesterol was accounted for by the

TABLE 16

RELATIONSHIP OF TOTAL CHOLESTEROL AND TOTAL LIPID IN PLASMA

	DAY:	0	0	20	20
	TREATMENT:[1]	TOTAL CHOLESTEROL	TOTAL LIPID	TOTAL CHOLESTEROL	TOTAL LIPID
Animal	Diet[2]				
1	1	122	201.8	120	279.1
2	1	132	214.8	110	223.9
3	1	148	271.9	102	104.0
4	1	129	238.4	94	154.8
5	2	138	215.6	148	475.5
6	2	122	224.3	129	365.1
7	2	130	261.1	124	217.7
8	2	114	172.3	126	203.0
9	3	120	227.0	115	175.6
10	3	122	232.6	142	199.7
11	3	140	206.6	138	200.8
12	3	122	199.3	124	193.5

$b = .104 \pm .033$

$r^2 = 0.32$

[1] mg/100 ml

[2] 1-Control, 2-15% Tallow, 3-15% Tallow + 5% Propionic Acid

relationship between plasma lipid and cholesterol. Regression analyses showed that the cholesterol values obtained were not significantly different between animals tested from start to finish of the 20 day experiment. The level of the total lipid estimated in the plasma samples appear to have little relationship to values obtained for total cholesterol.

3. Starvation Study: The effects of 12 hours starvation on plasma cholesterol concentrations are shown in Table 17.

Analysis of variance showed no significant differences in the plasma cholesterol levels of the same pigs when blood samples were taken while pigs were ad lib. fed or immediately after a 12 hour starvation period. The overall means for the 18 pigs before and after starvation were 111 mg and 110 mg per 100 ml respectively. The time of blood collection for cholesterol analysis reported in some experiments with swine has shown variability with collections made both after a period of fasting (Cox and Hale, 1960; Hutagalung et al, 1969; Calvert and Scott, 1974) and during feeding (Brooks et al, 1972; Hill and Silbernick, 1975; Aherne et al, 1976). Few studies have tested the effects of fasting on cholesterol levels. Experiments by Greer et al, (1966) demonstrated that serum cholesterol of swine increased in a linear manner for 2, 4 and 6 hours after feeding. However, Chen et al, (1976) reported that plasma cholesterol levels are not influenced significantly

TABLE 17

EFFECT OF STARVATION ON TOTAL PLASMA CHOLESTEROL CONCENTRATIONS[1]

TREATMENT		INITIAL[2]	12 HOURS STARVATION[2]
Animal	1	138	138
	2	124	120
	3	94	79
	4	95	94
	5	148	149
	6	94	98
	7	108	107
	8	120	121
	9	76	94
	10	118	124
	11	129	120
	12	136	139
	13	94	94
	14	120	98
	15	114	111
	16	94	108
	17	96	100
	18	96	94
Overall Mean		110.9	110.4
S.E.M.		1.38	
Significance		NS	

[1] Each value represents a mean of duplicate samples for each animal.

[2] Total plasma cholesterol (mg/100 ml).

by recent intakes of fat and therefore fasting before collection of blood samples is not necessary. These results are in agreement with those of the present study.

Summary

Twenty-seven crossbred pigs, weighing approximately 20 kg and previously fed a 15% tallow diet were allotted to three grower diets - control (C), 15% tallow (T), and a 15% tallow plus 5% propionic acid (T + PA). The pigs selected were those with the highest plasma cholesterol values. Plasma cholesterol levels were determined on days 0, 5, 10 and 20 of the 20 day experiment. Total plasma lipids were also determined from 12 pigs selected from Days 0 and 20 of the experiment. In addition 18 pigs from the initial population of 95 pigs were also selected to determine the effects of 12 hours starvation on plasma cholesterol levels. The results of this experiment demonstrated:

1. The inclusion of 5% propionic acid did not greatly reduce the plasma cholesterol levels of hypercholesterolemic type pigs fed a 15%T diet. The change from a 15%T to a C diet reduced cholesterol levels within 5 days.

2. The relationship of plasma cholesterol to plasma lipids accounted for only 32% of the variation encountered in the results. A simple regression on total lipid to cholesterol gave a value of b = .104.

3. No effects on cholesterol levels between fasted and non-fasted pigs were observed.

EXPERIMENT 5

1. Objectives: The objective of Experiment 5 was to determine the net rates of hepatic cholesterol synthesis by measuring the level of 7-dehydrocholesterol in blood of pigs dosed with a cholesterol inhibitor and fed diets with and without the inclusion of propionic acid.

2. Experimental Procedures

a. Animals, diets, and blood collection: Fifteen male castrated pigs from three litters of 3 to 4 weeks of age were fed the 15% tallow (T) starter diet shown in Table 18. Protein content of the diets containing 15% tallow were increased by 15% to adjust for reduced feed intake. Pigs were allotted to individual pens (0.7 m x 1.2 m) with partially slatted concrete floors. Pigs were allowed free access to feed and water. After a 4 day adjustment period the 12 heaviest pigs were bled by anterior vena cava puncture and plasma was collected as previously described (Experiment 1). Plasma samples were analyzed for total cholesterol and the eight pigs with the highest plasma cholesterol values were selected and randomly allotted on the following day to either a 15% tallow (T) or a 15% tallow plus 5% propionic acid (T + PA) starter diet. The four remaining pigs were allotted to a control (C) starter diet. The formulation and composition of these diets are

TABLE 18

FORMULATION AND COMPOSITION OF DIETS

EXPERIMENT 5

DIETS	C	15%T	15%T + 5%PA
Ingredients (percent)			
Wheat	25.0	19.0	14.0
Barley	25.0	15.0	14.0
Oat groats	25.0	20.0	19.0
Soybean meal (48.5 percent)	18.0	27.0	29.0
Stabilized tallow	3.0	15.0	15.0
Propionic acid[1]	--	--	5.0
Iodized salt	0.5	0.5	0.5
Calcium phosphate	1.5	1.5	1.5
Calcium carbinate	1.0	1.0	1.0
Vitamin-mineral premix[2]	1.0	1.0	1.0
Composition (calculated)			
Crude protein (percent)	19.1	20.7	20.7
Digestible energy (Kcal/kg)	3568	4191	4271
Calcium (percent)	0.75	0.77	0.77
Phosphorous (percent)	0.72	0.70	0.68

[1] Celanese Canada Limited, Edmonton (for composition see Table 3)

[2] The premix provided the following per killigram of diet: 120.0 mg Zinc; 10.0 mg Copper; 48.0 mg Manganese; 100.0 mg Iron; 45 IU Vitamin E; 7500 IU Vitamin A; 700 IU Vitamin D_3; 12 mg Riboflavin; 45 mg Niacin; 27 mg Calcium Pantothenate; 28 mcg Vitamin B_{12}; 150 mg Mecadox.

shown in Table 18. Pigs were allowed feed and water ad libitum for a period of 7 days. Animal weight and feed consumption records were maintained. The pigs were bled on the morning (9:00 - 9:30 A.M.) of day 5, 6 and 7 of the experimental period. Plasma 7-dehydrocholesterol concentrations were measured for all samples (see Methods of Chemical Analysis). Also plasma samples from day 5, 6 and 7 were analyzed for total cholesterol and plasma samples for day 7 were analyzed for calcium, phosphorus, glucose, blood urea nitrogen (B.U.N.), uric acid, total protein, albumin, bilirubin, alkaline phosphatase, latate dehydrogenase (L.D.H.) and serum glutamic oxaloacetic transaminase (S.G.O.T.) by a commercial laboratory[4] using a Technicon SMA 12/60 Autoanalyzer[5].

b. Dosing with AY-9944: Immediately after bleeding on day 5 (9:30 - 10:00 A.M.) the eight animals fed the tallow and tallow plus propionic acid diets were dosed with a solution of AY-9944[6] (467.9 mg dissolved in 60 ml of 0.9% saline) at a dose rate of approximately 19.5μ moles per kg live weight. Pigs were dosed using a stomach tube (polyvinyl chloride tubing, 0.5 O.D. x 45 cm) fitted with a three-way valve with two 12 ml syringes attached. The second syringe contained 5 to 6 ml 0.9% saline in order to facilitate washing of the

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[4] Dr. S. Hanson and Associates, Medical Laboratory, Edmonton, Alberta.

[5] Technicon Instruments Corporation, Tarrytown, New York.

[6] AY-9944 (trans-1,4-bis (2-chlorobenzylaminomethyl cyclohexane dihydrochloride), M.W. 464.3, Ayerst Laboratories, Montreal, Quebec.

stomach tube free of any remaining AY-9944 solution. The four remaining animals on the control diets were dosed with a second stomach tube in a similar manner but only 10 ml 0.9% saline was given. On day 7, immediately after blood samples were taken, animals which had received the AY-9944 were killed and incinerated.

Results and Discussion

1. Pig Performance: The mean values for ADG, ADF and EFC for each dietary treatment over the 7 day period are shown in Table 19. The pigs performed as expected and results were similar to those observed in Experiment 1.

2. Plasma 7-Dehydrocholesterol Studies: The effects of dietary treatment on the mean plasma 7-dehydrocholesterol levels for the three time periods measured are shown in Table 19. The data were analyzed using analysis of variance. Since the determined level of 7-dehydrocholesterol was zero for pigs fed the C diet at all three sampling periods and also at 0 hours for all treatment groups, there was no measure of statistical variation. Therefore, in order to handle the unequal variances Box's procedure as outlined by Gill (1970) was used. This procedure reduces the degrees of freedom thereby requiring a larger F-value.

The lack of any detectable level of plasma 7-dehydrocholesterol in animals which had not received the compound AY-9944 was consistent with values reported by

TABLE 19

PERFORMANCE AND BLOOD 7-DEHYDROCHOLESTEROL CONCENTRATIONS OF PIGS DOSED OR NOT DOSED WITH AY-9944[1]

ORAL DOSE:	SALINE ONLY	SALINE + AY-9944	SALINE + AY-9944		
TREATMENT:	C	15%T	15%T + 5%PA	S.E.M.	SIGNIFICANCE
Pig Performance[2]					
ADG (kg)	0.16	0.24	0.18	0.024	NS
ADF (kg)	0.48	0.36	0.30	0.060	NS
EFC (kg FEED/kg GAIN)	3.05[a]	1.51[b]	1.63[b]	0.191	***
Plasma 7-Dehydrocholesterol Concentrations[3]					
Time					
0 hours (pre-dose)	0.0	0.0	0.0	--[4]	--
24 hours (dosed)	0.0	23.4	14.8	--[4]	--
48 hours (dosed)	0.0	34.1	30.3	--[4]	--

[1] Each value represents a mean from duplicate values of 4 pigs.

[2] Performance is over a 7 day period.

[3] 7-dehydrocholesterol (mg/100 ml)

[4] Pooled S.E.M. computed from ANOVA was 1.08; S.E.M. for cells with means of 0.0 was 0, S.E.M. for cells with nonzero means was 1.69.

Horton et al (1971) who reported that 7-dehydrocholesterol could not be detected in the blood of rats that had not received the inhibitor AY-9944. Twenty-four hours after a single oral dose of AY-9944, 23.4 mg of 7-dehydrocholesterol per 100 ml plasma was detected in the plasma of pigs fed 15%T diets alone, which was approximately 58% higher than the level of 7-dehydrocholesterol (14.8 mg/100 ml) in the plasma of pigs fed the T + PA diets. After 48 hours the difference in plasma cholesterol levels between the two diets was less, with pigs fed T diets having levels 12% higher than pigs fed T + PA diets. The levels of 7-dehydrocholesterol were very highly significantly different ($P < 0.001$) between the dietary treatments at 24 and 48 hours.

The amount of 7-dehydrocholesterol in the blood after treatment with AY-9944 is representative of the cholesterol which would have been synthesized by the liver during the same time period had the inhibitor not been present (Horton et al, 1971; Dvornik et al, 1965). Therefore these results suggest that the rates of cholesterol synthesis occurring in animals fed dietary propionic acid is significantly lower than in animals fed tallow diets without propionic acid. These results suggest that propionic acid does have an inhibitory or competitive effect in the biochemical pathways of cholesterol formation.

3. Plasma Total Cholesterol and Profile Studies: The effects of dietary treatment and dosing with AY-9944 on the mean plasma total cholesterol and plasma constituents are shown in Table 20.

TABLE 20

PLASMA TOTAL CHOLESTEROL (0, 24 and 48 hours) AND

PLASMA CONSTITUENTS (48 hours)[1]

ORAL DOSE:	SALINE ONLY	SALINE + AY-9944	SALINE + AY-9944		
TREATMENT:	C	15%T	15%T + 5%PA	S.E.M.	SIGNIFICANCE
Total Cholesterol (mg/100 ml)					
0 hours	80[2]	78[2]	90[2]	--	--
24 hours	97[a]	65[b]	70[2]	3.6[3]	***
48 hours	129[a]	69[b]	82[b]	6.5	***
Calcium (mg/100 ml)	11.2	11.2	11.2	0.20	NS
Phosphorus (mg/100 ml)	8.7	8.3	8.2	0.36	NS
Glucose (mg/100 ml)	119	141	120	8.1	NS
B.U.N. (mg/100 ml)	15	18	18	1.1	NS
Uric Acid (mg/100 ml)	0.2	0.3	0.1	0.07	NS
Total Protein (g/100 ml)	4.8[a]	5.6[b]	5.2[ab]	0.16	*
Albumin (g/100 ml)	2.9	3.2	3.3	0.24	NS
Bilirubin (mg/100 ml)	0.05	0.08	0.02	0.026	NS
Alkaline Phosphatase (mU/ml)	374[a]	291[b]	304[b]	19.6	*
L.D.H. (mU/ml)	414	412	466	30.4	NS
S.G.O.T. (mU/ml)	59	103	117	29.8	NS

[1] Each value represents a mean of 4 pigs.

[2] Not included in statistical analyses.

[3] Comparison of C versus 15%T.

Due to inadequate sample size the values for plasma total cholesterol for 0 hours and the 24 hours T + PA were obtained from one determination of pooled plasma. These values were therefore not included in the statistical analysis. However a crude lsd test may be made using the pooled SEM (5.6) estimated from the 24 and 48 hours plasma combinations having four observations. The required lsd values $[(\sqrt{2})(2.13)(5.6) = 16.9]$ suggests there were no significant differences at 0 hours between any of the treatments and that for 24 hours after dosing the T + PA values would differ from the C but not from the T treatment values. Forty-eight hours after a single oral dose of AY-9944 plasma cholesterol concentrations were significantly lower in dosed than non-dosed pigs. The decrease in plasma cholesterol concentration after dosing with AY-9944 is consistent with reports in rats (Dvornik et al, 1963). However the levels of plasma cholesterol in pigs receiving AY-9944 did not appear to reflect the rates of synthesis as shown by the amount of 7-dehydrocholesterol present in the plasma after dosing with the inhibitor. Pigs fed T + PA diets had higher mean plasma cholesterol levels than pigs fed T diets at both 24 and 48 hours but these values were not significantly different.

Analyses of plasma constituents showed that for alkaline phosphatase pigs receiving the AY-9944 had significantly lower concentrations than C fed non-dosed pigs. Total protein concentrations were significantly higher in T fed dosed pigs and C fed non-dosed pigs only. No significant differences between treatments were observed for any of the other plasma constituents measured.

Summary

Twelve crossbred pigs, five weeks of age, were allotted to three starter diets - control (C), 15% tallow (T), 15% tallow plus 5% propionic acid (T + PA). Pigs fed T and T + PA diets were given a single oral dose of a cholesterol inhibitor AY-9944. Levels of plasma 7-dehydrocholesterol were determined at 0, 24 and 48 hours after dosing. Pig performance was also measured over a 7 day period. The results indicated:

1. Propionic acid in the diet of pigs appears to have an effect on the rates of cholesterol synthesis in the liver. Significant differences in the level of plasma 7-dehydrocholesterol were observed in animals dosed or not dosed with AY-9944 at both 24 and 48 hours.

2. No detectable level of plasma 7-dehydrocholesterol was observed in pigs not receiving the compound, AY-9944.

3. Plasma cholesterol levels were lower in pigs receiving AY-9944.

4. Pig performance was as expected and similar to previous experiments.

GENERAL DISCUSSION

A series of five experiments involving 237 pigs were undertaken to determine the effects, on blood and tissue cholesterol levels and performance, of including propionic acid with or without added fats, in diets of pigs. It was observed, in agreement with the data reported by the Agricultural Research Council (1967), that the addition of 10 or 15% tallow or 15% rapeseed oil to the diets resulted in a significant decrease in average daily feed intakes, an improved average daily gain, and a significant increase in feed conversion efficiency.

The results from the experiments demonstrated that feeding diets containing tallow generally resulted in increased plasma cholesterol levels but the increases were not always consistent with time or from experiment to experiment. Increasing the level of tallow in the diet from 10% to 15% (Experiment 3) increased plasma cholesterol levels but not significantly so. It is interesting to note that the addition of 15% rapeseed oil to the diet resulted in an increased plasma cholesterol equivalent to that of animals fed diets containing 15% tallow. This is in contrast to the general belief that unsaturated fats tend to produce lower cholesterol levels than saturated fats (Kritchevsky *et al*, 1956).

It is generally accepted that blood cholesterol concentrations in humans increase with age, with males

having higher levels than females. We did not observe these effects in any of the experiments performed with pigs. Our results are also in contrast to those of Tumbleson and Hutcheson (1976) who reported that female pigs had higher cholesterol levels than males. The lack of differences between males and females in our study could, in part, be explained by the fact that all the males used were castrated. This idea is supported by the results of Cox and Hale (1960) who observed that castration in pigs contributed to hypercholesterolemia.

Propionic acid in diets with or without added tallow tended to reduce plasma cholesterol levels. It is important to observe that in Experiment 1 the reduction obtained in plasma cholesterol levels was greater in diets where tallow was not added. The ratio of propionyl-CoA units to acetyl-CoA units present in the blood may be an important factor in determining the degree of inhibition of the enzyme beta-hydroxymethylglutaryl-CoA synthase. This effect can be viewed in the same manner as the classical example of competitive inhibition of succinate dehydrogenase by malonate and other dicarboxylic acids (Lehniger, 1970). In this reaction malonate and succinate present in the ratio of 1:50 inhibits succinate dehydrogenase activity by 50% regardless of their absolute concentrations. The increased presence of acetyl units made available from high tallow feeding would alter the ratio of acetyl:propionyl units thereby potentially reducing the effectiveness of the inhibition mechanism. Propionic acid added alone to control

diets (Experiment 1) exhibited the largest decreases in plasma cholesterol levels whereas as propionic acid added to 15% tallow diets did not have much effect.

According to the World Health Organization Report (1974), in man, the liver can deal with 4.5 g of free acid per hour. Considering the overall performance data from Experiment 1, and assuming complete absorption of propionic acid, pigs fed 5% propionic acid diets and 10% tallow plus 5% propionic acid diets were making available on the average 4.8 g and 4.2 g of free acid to the liver per hour respectively. Since the liver in man can deal with 4.5 g of free acid per hour it is interesting to speculate whether the levels in Experiment 1 were high enough to allow for the required amount of propionyl-CoA needed for inhibition purposes. It would have been interesting to examine whether or not feeding higher levels of propionic acid might improve the ratios of acetyl-CoA:propionyl-CoA. Higher levels of available propionyl-CoA units could thereby improve the amount of inhibition obtained, especially so, in diets without added fat.

Tissue cholesterol levels were generally not affected by the addition of tallow or propionic acid to the diet. Only kidney tissue values reflected the anticipated increases with tallow feeding and decreases with propionic acid feeding. Overall plasma and tissue cholesterol levels did not reflect increased rates of synthesis on high fat diets. Measurements of cholesterol excretion patterns may have given some further insight as to what was happening

to the cholesterol obtained from the increased rates of synthesis. In addition, excretion patterns of cholesterol may also have been altered by propionic acid feeding to compensate for reduced rates of synthesis. According to Marsh et al (1972) total body cholesterol is maintained at constant levels when the counterbalancing mechanisms of absorption, excretion (via the intestinal mucosa and bile) and synthesis are in harmony. They suggested that any deviation will eventually result in an expansion or reduction in tissue pools of cholesterol. As a result rates of synthesis of cholesterol may have been reflected in changes in excretion patterns.

Studies in man by Quintao et al (1971) showed that there was no close relationship between changes in the tissue levels and blood plasma levels of cholesterol. Blood plasma may therefore be not a good indicator of changing cholesterol pool size and changing rates of synthesis. The results obtained in Experiment 5, where inhibition of cholesterol was acquired with AY-9944, substantiate that blood levels may not be indicative of the amount of synthesis that is occurring. The different observed concentrations of plasma 7-dehydrocholesterol in pigs fed tallow compared to pigs fed tallow plus propionic diets suggested that propionic acid does have an effect on the rates of cholesterol synthesis and may not be reflected in plasma levels of cholesterol. This effect could have contributed to the results previously observed with propionic acid feeding in Experiments 1 and 4.

There is very little influence of dietary cholesterol intake on plasma cholesterol levels in swine. Cholesterol intakes under normal dietary conditions are almost negligible according to Marsh et al (1972) who reported that only 4.7% of the cholesterol fed per day to pigs on a low fat diet was absorbed. A more detailed study of the total body cholesterol pool should be attempted before one can fully evaluate the effectiveness of propionic acid in reducing rates of cholesterol synthesis.

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APPENDIX A

DETERMINATION OF TOTAL CHOLESTEROL IN BILE

Reagents:

Hexane (distilled)
Concentrated HCl
Isopropanol (reagent grade)

Procedure:

1. Pipet 0.5 ml bile into 13 x 100 mm teflon screw-cap culture tubes.
2. Add 1 ml hexane.
3. Add 3 ml distilled water. Mix.
4. Add 2 drops concentrated HCl. Mix.
5. Centrifuge for 5 minutes at 2000 rpm.
6. Transfer 0.5 ml aliquot of the hexane layer into a 20 x 150 mm teflon screw-cap culture tube.
7. Add 9.5 ml isopropanol. Mix. Let stand for 10 minutes and centrifuge for 5 minutes at 1000 rpm.
8. Using a pasteur pipet transfer approximately 2 ml of the supernatant to autoanalyzer cups for analysis.

APPENDIX B

ESTIMATION OF TOTAL LIPIDS IN BLOOD PLASMA

Reagents:

45% Potassium Hydroxide (KOH)
Distilled: Methanol (MeOH); N-pentane; H_2O
HCl (concentrated)
Standard $C_{17}COOH$ (Heptadecanoic Acid) (0.124 mg/ml methanol)
Methylation reagent (35% BF_3, 20% Pentane, 45% methanol).

Procedure:

1. 2 ml of plasma was pipetted into 20 x 150 mm teflon screw-cap culture tubes. 10 ml of MeOH and 2 ml KOH was added and the solution mixed. The tubes were capped and boiled in a water bath for 30 minutes. Allow to cool.
2. Add 5 ml standard $C_{17}COOH$. Mix.
3. Mix in 2 ml concentrate HCl or until acidic. Shake and allow phases to separate. Remove upper phase to a new tube.
4. Evaporate to dryness under N_2.
5. Add 10 ml methylation reagent. Heat gently on a steam bath for approximately 15 minutes. Allow to cool.
6. Add 15 ml distilled water. Mix and let phase separate. Remove upper phase. Re-extract with a few mls of pentane.
7. Concentrate under N_2 for gas chromatography.

$$\frac{\text{Weight } C_{17}COOH}{\text{Area } C_{17}COOH} \times \text{Area}_{sum} = \text{weight of total lipid}$$

The conditions that prevailed during gas chromatography were

Column (1) glass - pyrex - 360 cm x 5 mm
(2) packed with 10% 5CP Chromosorb W 80/100
(3) temperature: 215°C

Inlet and flame detector temperature = 250°C - 290°C.
Nitrogen and hydrogen flow rates were 15 ml/minute and 10 ml/minute respectively.
Air flow rate was 15 ml/minute.

APPENDIX C

DETERMINATION OF FATTY ACID PATTERNS IN TALLOW OR OIL

Reagents:

Distilled: pentane, water, benzene
2:1 Chloroform: Methanol ($CHCl_3$:MeOH)
Methylation reagent (35% BF_3, 20% pentane, 45% methanol)

Procedure:

1. Add 0.5 - 1.0 g fat or 0.5 - 1.0 ml oil to teflon capped tube. Extract with approximately 30 ml $CHCl_3$:MeOH mixture (2:1).
2. Evaporate to dryness under N_2 in a water bath (initial temperature less than 50°C).
3. Add approximately 10 - 15 ml methylation reagent. (35% BF_3 solution (10% BF_3 in MeOH), 20% n-pentane and 45% methanol).
4. Close cap and heat in boiling water bath for 40 - 60 minutes. Shake occasionally.
5. Cool tube to room temperature, open and carefully add approximately 5 ml distilled H_2O and 10 ml pentane. Shake briefly.
6. Remove pentane (upper layer) with a pasteur pipette and store under N_2 in glass vials with teflon lined caps.
7. Purify the methyl esters by TLC. Develop the plate in benzene to the top (approximately 1 1/2 hours). Identify methyl esters under short wave UV light. Scrape the methyl esters and extract with $CHCl_3$:MeOH (2:1). Evaporate under N_2 in glass vials with teflon lined caps.
8. Dissolve in small amount of pentane and run on GC.

Fatty Acid Composition of Tallow and Rapeseed Oil Samples

The results of the fatty acid patterns of one rapeseed oil ('Tower') and two stabilized tallow samples are shown in Table 21. The percentage composition by weight of the individual fatty acids in the 'Tower' rapeseed oil sample are in agreement with results of Aherne et al (1976) and Slinger (1977). The values obtained for the two tallow samples were consistent with values of beef fat presented by Mayes (1973). The degree of saturation and unsaturation of the animal and vegetable fats used in the experiments in this study were consistent in composition with similar fats or oils used.

TABLE 21

FATTY ACID PATTERNS OF TALLOW AND RAPESEED OIL IN PERCENTAGE BY WEIGHT OF FATTY ACIDS

Fatty Acid	TALLOW A	TALLOW B	RAPESEED OIL 'TOWER'
Myristic (C14:0)[1]	1.6	2.3	0.3
Palmitic (C16:0)	22.2	24.1	4.8
Palmitoleic (C16:1)	3.3	3.4	0.4
Heptadecanoic (C17:0)	1.2	1.4	--
Stearic (C18:0)	21.2	21.4	2.7
Oleic (C18:1)	43.2	40.0	56.2
Linoleic (C18:2)	4.0	3.4	24.8
Linolenic (C18:3)	0.5	0.5	7.3
Arachidic (C20:0)	--	--	0.6
Eicosenoic (C20:1)	--	--	1.6
Behnic (C22:0)	--	--	0.5
Erucic (C22:1)	--	--	0.3
Others	2.8	3.5	0.5

[1] Numbers before and after colon represent the number of carbon atoms and double bonds respectively.

APPENDIX D

DETERMINATION OF 7-DEHYDROCHOLESTEROL

Reagents:

30% Potassium hydroxide (KOH)
Petroleum ether (b.p. 30 - 60°C)
Distilled ethanol
Concentrated glacial acetic acid
Leibermann-Burchard Color Reagent

Reagent preparation:

1. Color reagent: Mix 220 ml of cold acetic anhydride with 200 ml glacial acetic acid (room temperature) in an amber glass bottle. Add 30 ml cold concentrated H_2SO_4 (Mixture good for 6 months at 4°C).

Procedure:

1. Saponification:

   a. Pipet duplicate samples of plasma into 20 x 150 mm teflon screw-cap tubes. Saponify for 1 hour at room temperature with 5 ml 30% KOH.

2. Extraction:

   a. Add 2 ml ethanol. Add 8 ml petroleum ether, mix and let phases separate. Remove upper phase to second tube. Re-extract with another 8 ml petroleum ether. Remove upper phase.

   b. Evaporate second tube to dryness in a water bath (approximately 60°C) under N_2.

   c. Dissolve the residue in 0.5 ml glacial acetic acid.

   d. Add 3 ml of the color reagent. Shake and read at A 625 nm after 2 minutes.

Standard 7-dehydrocholesterol: 106.2 mg 7-dehydrocholesterol dissolved in 100 ml distilled ethanol.

Blank: 0.5 ml glacial acetic acid
3 ml color reagent